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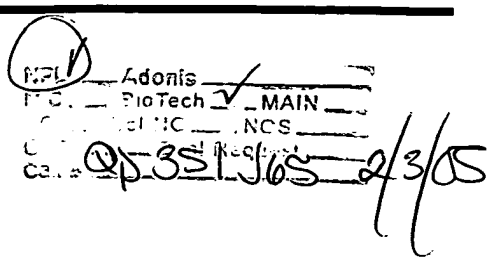

1) 10773013 PMID: 10893637
Induction of apoptosis in BPH stromal cells by adenoviral-mediated overexpression of caspase-7.
Marcelli M; Shao T C; Li X; Yin H; Marani M; Denner L; Teng B; Cunningham G R
Departments of Medicine, Baylor College of Medicine and VA Medical Center, Houston, Texas, USA.
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Induction of caspase-3-like protease may mediate delayed neuronal death in the hippocampus after transient cerebral ischemia.
Chen J; Nagayama T; Jin K; Stetler R A; Zhu R L; Graham S H; Simon R P
Department of Neurology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213, USA.
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MINH TAM DAVIS
ART UNIT 1642, ROOM 3A24, MB 3C18
272-0830

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Induction of Caspase-3-Like Protease May Mediate Delayed Neuronal Death in the Hippocampus after Transient Cerebral Ischemia

Jun Chen,[1] Tetsuya Nagayama,[1] Kunlin Jin,[1] R. Anne Stetler,[1,2] Raymond L. Zhu,[1] Steven H. Graham,[1,2] and Roger P. Simon[1]

[1]Department of Neurology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213, and [2]Neurology Service, Department of Veterans Affairs Medical Center, Pittsburgh, Pennsylvania 15261

Delayed neuronal death after transient cerebral ischemia may be mediated, in part, by the induction of apoptosis-regulatory gene products. Caspase-3 is a newly characterized mammalian cysteine protease that promotes cell death during brain development, in neuronal cultures, and in other cell types under many different conditions. To determine whether caspase-3 serves to regulate neuronal death after cerebral ischemia, we have (1) cloned a cDNA encoding the rat brain caspase-3; (2) examined caspase-3 mRNA and protein expression in the brain using *in situ* hybridization, Northern and Western blot analyses, and double-labeled immunohistochemistry; (3) determined caspase-3-like activity in brain cell extracts; and (4) studied the effect of caspase-3 inhibition on cell survival and DNA fragmentation in the hippocampus in a rat model of transient global ischemia. At 8–72 hr after ischemia, caspase-3 mRNA and protein were induced in the hippocampus and caudate-putamen (CPu), accompanied by increased caspase-3-like protease activity. In the hippocampus, caspase-3 mRNA and protein were predominantly increased in degenerating CA1 pyramidal neurons. Proteolytic activation of the caspase-3 precursor was detected in hippocampus and CPu but not in cortex at 4–72 hr after ischemia. Double-label experiments detected DNA fragmentation in the majority of CA1 neurons and selective CPu neurons that overexpressed caspase-3. Furthermore, ventricular infusion of Z-DEVD-FMK, a caspase-3 inhibitor, decreased caspase-3 activity in the hippocampus and significantly reduced cell death and DNA fragmentation in the CA1 sector up to 7 d after ischemia. These data strongly suggest that caspase-3 activity contributes to delayed neuronal death after transient ischemia.

Key words: cerebral ischemia; caspase-3; apoptosis; cysteine protease; neuronal death; neuron

Transient global ischemia results in delayed neuronal death in selectively vulnerable brain regions such as the hippocampal CA1 sector and caudate-putamen (Pulsinelli et al., 1982). A number of recent studies suggest that cell death in this setting involves apoptosis–an active and genetically controlled cell suicide process. Histological and biochemical characteristics of apoptosis are present in dying neurons after ischemia (MacManus et al., 1993; Kihara et al., 1994; Nitatori et al., 1995), and inhibition of new protein synthesis protects CA1 neurons after ischemia (Goto et al., 1990; Papas et al., 1992). Several apoptosis-regulatory genes are found to be induced in ischemic cells. Bax, a bcl-2 homolog that effects apoptosis, is upregulated in neurons destined to die after global ischemia (Krajewski et al., 1995; Chen et al., 1996), whereas the apoptosis-suppressor gene bcl-2 is expressed in neurons that survive ischemia (Shimazaki et al., 1994; Chen et al., 1997a). Furthermore, bcl-2 overexpression in rodent brain reduces ischemic injury (Martinou et al., 1994; Linnik et al., 1995; Lawrence et al., 1996). Taken together, these observations suggest that endogenously induced apoptosis-regulatory genes may play a role in determining the fate of ischemic neurons.

The interleukin-1β-converting enzyme (ICE) family of cysteine proteases, now referred to as caspases, is another group of apoptosis-regulatory genes that may play a role in ischemic brain injury (Bredesen, 1995). The ICE family, consisting of at least 11 members (caspases-1–11), represents mammalian homologs of ced-3, an essential cell death gene in *Caenorhabditis elegans* (Yuan et al., 1993; Fernandes-Alnemri et al., 1994; Xue et al., 1996). When overexpressed, the caspases trigger apoptosis in cultured cells (Miura et al., 1993). Among the identified caspases, caspase-3 (also termed cpp32, Yama, or Apopain) exhibits the highest sequence homology to ced-3 (Tewari et al., 1995). Caspase-3 is a potent effector of apoptosis triggered via several different pathways in a variety of mammalian cell types (for review, see Alnemri et al., 1996). Caspase-3 promotes neuronal death during brain development (Kuida et al., 1996). In neuronal cultures, induction of caspase-3-like protease promotes apoptosis induced by withdrawal of trophic support, K^+ deprivation, or glutamate excitotoxicity (Deshmukh et al., 1996; Schulz et al., 1996; Du et al., 1997; Keane et al., 1997; Ni et al., 1997). Moreover, inhibition of ICE- or caspase-3-like protease provides protection in rodent brains subjected to focal ischemia or direct excitotoxic insults (Hara et al., 1997) or to traumatic brain injury (Yakovlev et al., 1997). Thus, caspase-3 and related caspases could be important neuronal death effectors in the brain under certain pathological conditions.

We, therefore, characterized the regional and temporal profiles

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Correspondence should be addressed to Dr. Jun Chen, Department of Neurology, S506-Biomedical Science Tower, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

of caspase-3 gene expression in the rat brain at both mRNA and protein levels after transient global ischemia. Then we determined whether caspase-3-like protease activity is altered in the brain after ischemia and how the altered enzymatic activity correlates with regional vulnerability to ischemia. Lastly, by inhibiting caspase-3-like activity in the hippocampus *in vivo*, we investigated the role of induction of this important cell death gene in determining the fate of ischemic neurons.

MATERIALS AND METHODS

Animal model of transient global ischemia

Experiments were performed on a total of 238 male Sprague Dawley rats weighing 300–350 gm (Hilltop Sprague Dawley, Scottdale, PA). Transient global ischemia (15 min) was induced in isoflurane-anesthetized rats using a previously described method (Pulsinelli et al., 1982), with modifications (Chen et al., 1996). Blood pressure, blood gases, and blood glucose concentration were monitored and maintained in the normal range throughout the experiments. Rectal temperature was continuously monitored and kept at 37–37.5°C using a heating pad and a temperature-regulated heating lamp. Brain temperature was monitored by a 29 ga thermocouple implanted in the left striatum and kept at 36.4 ± 0.2°C during ischemia and at 37–37.5°C thereafter. An electroencephalogram (EEG) was monitored in all animals to ensure isoelectricity within 10 sec after carotid artery occlusion. A sham operation was performed in additional animals using the same anesthesia and surgical exposure procedures except that the arteries were not occluded; these brains were used as nonischemic controls.

cDNA cloning

Construction of a cDNA library. Hippocampi were dissected from rat brains subjected to 15 min of ischemia followed by 8, 24, or 72 hr of reperfusion (three brains per time point). Polyadenylated mRNAs were isolated from the hippocampi using a fast track mRNA isolation kit (Invitrogen, San Diego, CA) and were used as templates for cDNA synthesis. An ischemic brain cDNA library was constructed using a SuperScript plasmid system for cDNA synthesis and a plasmid cloning kit according to the manufacturer's instructions (Gibco BRL, Gaithersburg, MD). In brief, first-strand cDNA was synthesized using the oligo-dT_{12}-*Not*I primer adapter and SuperScript II reverse transcriptase (RT); second-strand synthesis was catalyzed by *Escherichia coli* DNA polymerase I in combination with *E. coli* RNase H and *E. coli* DNA ligase. Double-stranded DNA was restricted with *Not*I and *Sal*I, selected on a cDNA size fraction column to include molecules of >500 bp, ligated into plasmid pSPORT 1, and transformed into *E. coli* DH5a. The constructed cDNA library was examined by the color-selection method using isopropyl-1-thio-β-D-galactoside-X-gal and titer measurement after amplification. The titer of the amplified library was 10^{11} pfu/ml.

Isolation of a cDNA encoding caspase-3. In preliminary studies, a 345 bp cDNA fragment encoding the rat caspase-3 was generated by RT-PCR using primers designed according to the conserved sequences in human and mouse caspase-3 (Fernandes-Alnemri et al., 1994; Tewari et al., 1995). This cDNA fragment was labeled with [^{32}P]dATP using random primers and SuperScript II RT (Gibco BRL) and then was purified using a NucTrap probe purification column (Stratagene, La Jolla, CA). To obtain a cDNA containing the open reading frame encoding the caspase-3 protein, we used the labeled cDNA probe to screen the ischemic cDNA library. Colonies of the cDNA library were plated onto 137 mm filters, denatured, and neutralized. The filters were hybridized in 50% formamide-containing 5× saline-sodium phosphate-EDTA buffer, 1% SDS, 2× Denhardt's solution, and 5% dextran sulfate with the ^{32}P-labeled caspase-3 cDNA probe (5×10^6 cpm/ml) at 42°C for 18 hr. The resulting positive clones were sequenced on both strands using the dideoxy chain termination technique (Sequenase II; United States Biochemicals, Cleveland, OH). Sequence analysis was performed using MacVector software (International Biotechnologies, New Haven, CT) and aligned using the BLAST program (www.ncbi.nlm.nih.gov).

In vitro *transcription and translation.* To confirm that the cDNA contains the full open reading frame, we performed *in vitro* transcription and translation to detect its protein product. Transcription was performed using an RNA transcription kit according to the manufacturer's instructions (Stratagene). In brief, 1 μg of linearized plasmid DNA was incubated at 37°C for 1 hr in a mixture containing 40 mM Tris-HCl, pH 8.0, 8 mM $MgCl_2$, 2 mM spermidine, 50 mM NaCl, 40 mM DTT, 2 mM dNTP, and 10 U of T7 RNA polymerase. After DNA digestion using 200 U of RNase-free DNase (Promega, Madison, WI), RNA was extracted from the reaction mixture with phenol and chloroform followed by precipitation with 0.5 volume of ammonium acetate and 2.5 volumes of ethanol. *In vitro* translation was then performed at 30°C for 1 hr in a reaction system containing 100 ng of extracted RNA, 2 μl of translation reaction mixture (Boehringer Mannheim, Indianapolis, IN), 10 μl of rabbit reticulocyte lysate, 100 mM potassium acetate, and 1 mM magnesium acetate in the presence of [^{35}S]methionine (>800 Ci/mmol; NEN, Boston, MA). Translated protein was electrophoresed in a 12% SDS-polyacrylamide gel and detected by autoradiography.

Northern blot analysis

Total RNA was isolated from the hippocampi of brains subjected to 15 min of ischemia followed by 8 and 24 hr of reperfusion (n = 3 per time point) or brains 24 hr after sham operation (n = 3) as described previously (Chen et al., 1996). RNA was electrophoresed on a 1.0% agarose–formaldehyde gel, blotted onto Hybond-N membrane (Amersham, Arlington Heights, IL), and prehybridized for 2 hr at 42°C. The membranes were subsequently hybridized with the rat caspase-3 cDNA probe for 24 hr at 42°C. The ^{32}P-labeled cDNA probe was prepared using the random primer method. cDNA inserts were released from the plasmid with restriction enzyme digestion and purified using the Gene Clean kit (BIO 101, La Jolla, CA). Approximately 25 ng of cDNA inserts was dissolved in 20 μl of distilled water in a microcentrifuge tube, heated for 5 min in a boiling water bath, and then immediately cooled on ice. Two microliters of a reaction mixture containing 0.5 mM each of dATP, dTTP, and dGTP; 10 μl of random primer buffer (Gibco BRL); 50 μCi of [α-^{32}P]dCTP; and 3 U of the large fragment of DNA polymerase I were added to the cDNA preparation and incubated at room temperature for 1 hr. The labeled probe was purified using the NucTrap probe purification column (Stratagene). Labeling efficiency of the probe was determined by measuring percentage incorporation of the radioisotope. After the hybridization and washing procedures, autoradiography was performed at −80°C overnight with an intensifying screen. Autoradiograph signals were quantified by a gel densitometric scanning program using the Microcomputer Imaging Device (MCID) image analysis system. To control for variation in the amount of total RNA in different samples, we stripped off the original probe in a solution containing 0.1× SSC and 0.5% SDS at 100°C for 15 min. The blot was rehybridized with an oligodeoxynucleotide probe (5′-ACGGTATCTGATCGTCTTCGA-ACC-3′) corresponding to 18S RNA. All densitometric values for caspase-3 were normalized to values for 18S RNA obtained on the same lane.

In situ *hybridization*

The ^{35}S-labeled single-stranded RNA probe was prepared from plasmids containing the rat caspase-3 cDNA inserts. For the preparation of the antisense probe, the plasmid was linearized by *Xho*I digestion. A product complementary to the rat caspase-3 mRNA was transcribed by the T3 RNA polymerase in the presence of 125 μCi of ^{35}S-UTP (NEN) using an *in vitro* transcription kit according to the manufacturer's instructions (Stratagene). For the sense probe, the plasmid was linearized by *Pst*I and subsequently transcribed using the T7 RNA polymerase (Stratagene). The transcription reaction was performed for 45 min at room temperature, and the cDNA template was then digested for 10 min at 37°C using DNase I (10 U) in the presence of tRNA (20 μg). The RNA probe was extracted from the reaction mixture using phenol and chloroform followed by precipitation with 0.5 volume of ammonium acetate and 2.5 volumes of ethanol. After centrifugation, the pellet was rinsed with cold graded ethanol, air dried, and resuspended in 50 μl of 10 mM DTT. Labeling efficiency of the probe was determined by measuring percentage incorporation of the radioisotope. Frozen sections from ischemic, sham-operated, or naive control brains were prepared as described previously (Chen et al., 1996). Coronal sections at the levels of the dorsal hippocampus (anteroposterior, −3.5 to −4.0 mm from the bregma) were selected and processed for *in situ* hybridization. Neuronal degeneration at these levels after 15 min of global ischemia has been characterized and described elsewhere (Chen et al., 1996, 1997a, 1998). The sections were treated under RNase-free conditions with 4% paraformaldehyde in PBS for 20 min, rinsed twice for 5 min in PBS, and then acetylated twice for 5 min with acetic anhydride in 0.1 M triethanolamine-HCl, pH 7.5. After washing in PBS for 5 min and saline for 5 min, the sections were dehydrated in graded ethanol and air-dried. The sections were hybridized with the labeled RNA probe (1×10^7 cpm/ml) in a hybridization

cocktail for 18 hr at 55°C. The slices were then washed twice for 10 min in 2× SSC (300 mM sodium chloride and 30 mM sodium citrate, pH 7.4), once for 2 hr in 0.1× SSC at 50°C, and then twice for 10 min in 0.5× SSC at room temperature. They were then dehydrated, air dried, and autoradiographed onto Kodak SB-5 film for 3 weeks. Control and ischemic brain slides were processed together and developed on the same film. Relative changes in regional mRNA expression were semiquantitated using the MCID system (St. Catharine's, Ontario, Canada) as described previously (Chen et al., 1998). Cellular localization of the labeled mRNA was evaluated by coating slides with Kodak NTB-2 emulsion. Sections were exposed at 4°C for 5 weeks, developed in Kodak D-19, and counterstained with cresyl violet.

Western blot analysis

Animals were killed at 4, 8, 24, or 72 hr after 15 min of ischemia or 24 hr after sham operation (n = 4 per experimental condition). The hippocampus, striatum, and cortex were separately dissected, homogenized, and lysed. The lysates were cleared by centrifugation at 14,000 × g for 30 min at 4°C. The protein was denatured in SDS gel-loading buffer (100 mM Tris-HCl, 200 mM dithiothreitol, 4% SDS, 0.2% bromophenol blue, and 20% glycerol) at 100°C for 6 min and then separated on 12% SDS-polyacrylamide gels (40 μg per sample). Immunoblotting was performed as described previously (Chen et al., 1996), using a chemiluminescent detection system (Clontech, Palo Alto, CA). The antibody used to detect the caspase-3 protein was a custom-made rabbit polyclonal antibody (Biosynthesis, Dallas, TX) raised against the deduced C-terminal sequence (NH_2-QACRGTELDCGIETD-COOH) of the rat caspase-3 larger active form p17. The working dilution for caspase-3 antibody in the present study was 1:2000. The synthesized peptide for immunization was also used in preabsorption experiments to confirm the specificity of the detected immunoreactivity. This was done by incubating the peptide (5 μg/ml) with the primary antibody for 30 min at 37°C before the immunoblotting was performed. For immunoblotting to detect poly(ADP-ribose) polymerase (PARP), a monoclonal antibody against the C-terminal part of the DNA binding domain of PARP was used at a working dilution of 1:5000, as suggested by the manufacturer (Biomol, Plymouth Meeting, PA). A purified bovine PARP protein was used in preabsorption experiments to confirm the specificity of PARP immunoreactivity. Immunoreactivity for caspase-3 or PARP on each individual lane of the blots was quantified by a gel densitometric scanning program using the MCID image analysis system.

Immunohistochemistry

Animals were anesthetized with 8% chloral hydrate at 4, 8, 24, or 72 hr after 15 min of ischemia or 24 hr after sham operation (n = 4 per time point). They were then perfused with 200 ml of heparinized 0.9% saline followed by 500 ml of 4% paraformaldehyde in 0.1 M PBS, pH 7.4. The brains were removed, immersed in 4% paraformaldehyde for 5 d, and processed for paraffin embedding and cutting (6 μm thick) on a rotary microtome. Coronal sections at the levels of dorsal hippocampus and midcaudate (anteroposterior, +0.2 mm from the bregma) were selected and processed for immunohistochemical staining. The sections were deparaffinized in xylene, rehydrated through graded ethanol, and pretreated with protease K (10 μg/ml for 10 min). After three 10 min washes in PBS, the sections were microwaved in sodium citrate buffer, pH 6.0, for 5 min before immunostaining. Immunohistochemical staining for caspase-3 was performed using the same antibody used for Western blot analysis. Briefly, after the preblocking step using the rabbit preimmunizing serum, sections were incubated overnight at room temperature in the primary antibody diluted 1:1000 in PBS, pH 7.4, containing 2% goat serum, 0.2% Triton X-100, bovine serum albumin (5 mg/ml), and 0.2% glycine. Sections were washed in PBS three times for 10 min each and then incubated for 1 hr at room temperature at a 1:2500 dilution with goat anti-rabbit Cy3.18 immunoconjugate (Jackson ImmunoResearch, West Grove, PA). Sections were then washed in PBS four times for 15 min each on a orbital shaker (model 361; Fisher Scientific, Houston, TX), mounted in gelvatol, and coverslipped. A Zeiss light microscope equipped for epifluorescent illumination was used for observation. For the assessment of nonspecific immunostaining, alternating sections from each experimental condition were incubated without the primary antibody or, in some cases, with antibody that was preabsorbed with the synthetic caspase-3 peptide (8 μg/ml) for 30 min at room temperature before immunohistochemistry. Immunoreactivity was compared in sections from animals killed 24 hr after ischemia, with or without preabsorption of the primary antibody.

Detection of DNA fragmentation

In situ detection of DNA fragmentation in brain cells after ischemia or after sham operation was performed using terminal deoxynucleotidyl transferase-mediated biotin-dUTP nick-end labeling (TUNEL) as described previously by Gavrieli et al. (1992), with modifications (Chen et al., 1997b). For double-label experiments, paraffin-imbedded sections, at the levels of dorsal hippocampus and midcaudate, obtained 24 and 72 hr after ischemia and 24 hr after sham operation (n = 4 per experimental condition) were used. The sections were pretreated as described above and then incubated at 37°C for 90 min in 1× terminal deoxynucleotidyl transferase (TdT) buffer containing 100 U/ml TdT and 20 nmol/ml biotin-conjugated 16-dUTP (Boehringer Mannheim). After three washes in PBS (10 min/wash), the sections were incubated at room temperature for 15 min in fluorescein-avidin D cell sorting (DCS) (cell sorting grade; Vector Laboratories, Burlingame, CA) diluted in PBS at 8 μg/ml. Sections were then processed for caspase-3 immunohistochemistry as described above, and all steps were performed in the dark. Sections were examined by fluorescence microscopy using excitation/emission wavelengths of 550/565 nm (red) and 495/515 nm (green–yellow) for caspase-3 and TUNEL, respectively.

Activities of caspase-3- and ICE-like proteases

Measurement of caspase-3- and ICE-like protease activity in brain cell extracts was performed as originally described (Enari et al., 1996) with the slight modifications suggested by Clontech. The animals were anesthetized using 8% chloral hydrate and decapitated. Brains were quickly removed. Tissues were dissected separately from the hippocampus, striatum, cortex, or cerebellum of brains at 1, 4, 8, 24, and 72 hr after ischemia, at 24 hr after sham operation, or from naive animals (n = 4–5 per experimental condition). Protein extracts were prepared on ice by Dounce homogenization of tissues in a lysis buffer containing 25 mM HEPES, 5 mM EDTA, 1 mM EGTA, 5 mM $MgCl_2$, 5 mM DTT, and 10 μg/ml each of pepstatin, leupeptin, aprotinin, and PMSF (Sigma, St. Louis, MO). Cell lysate was centrifuged at 15,000 rpm for 10 min, and the supernatant was used for the enzymatic assay. One hundred micrograms of the extracted proteins were incubated for 1 hr at 37°C with the reaction buffer (25 mM HEPES, pH 7.5, 10% sucrose, 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate, 5 mM DTT, and 5 mM EDTA) in a total volume of 150 μl containing 25 μM colorimetric peptide substrate (Biomol). The following two substrates were used for the assays: acetyl-Asp-Glu-Val-Asp-*p*-nitroanilide (Ac-DEVD-*p*NA) for caspase-3-like protease activity and acetyl-Tyr-Val-Ala-Asp-*p*-nitroanilide (Ac-YVAD-*p*NA) for ICE-like protease activity. Enzyme-catalyzed release of *p*-nitroanilide was measured at 405 nm in a microtiter plate reader (Molecular Devices, Palo Alto, CA). One unit of protease activity corresponds to the caspase-like activity that cleaves 1 pmol of *p*NA per minute at 37°C at saturating substrate concentrations. In certain experiments, the extracted protein sample was diluted in the reaction buffer and first incubated with inhibitors for caspase-3 (DEVD-CHO) and ICE (YVAD-CHO) at room temperature for 30 min (Lazebnik et al., 1994; Nicholson et al., 1995).

In vivo *inhibition of caspase-3-like protease*

N-Benzyloxycarbonyl-Asp(OMe)-Glu(OMe)-Val-Asp-(OMe)-fluoro-methylketone (Z-DEVD-FMK) (Enzyme Systems Products, Livermore, CA), a peptide methylketone caspase-3 inhibitor (Nicholson et al., 1995; Rodriguez et al., 1996), was dissolved in DMSO at a concentration of 18.75 mg/ml and further diluted 1:25, 1:75, or 1:225 in mock CSF. Peptide infusions were performed using a 10 μl Hamilton syringe (Hamilton, Reno, NV) through a preimplanted 21 ga cannula in the left ventricle (from the bregma: anteroposterior, −0.8 mm; lateral, 1.5 mm; depth, 3.5 mm). Each animal received three ventricular infusions of 2 μl each over a 5 min time period 30 min before ischemia and 2 and 24 hr after ischemia, except as indicated otherwise. The resulting peptide doses for infusion treatment were 0.057 μg × 3, 1.5 μg × 3, 0.5 μg × 3, and 0.167 μg × 3 per animal. Infusions of diluted DMSO (0.04%) served as vehicle controls. All treatments were assigned to animals in a randomized and blinded manner. Brain and rectal temperatures were monitored in all animals before, during, and up to 2 hr after ischemia. The rectal temperature was also measured at 24, 48, and 72 hr after ischemia.

The doses of Z-DEVD-FMK used in the present study were suggested by the focal ischemia studies of others (Hara et al., 1997) and determined by a series of experiments in which the effectiveness of this peptide to inhibit caspase-3 activity in the hippocampus after ischemia was evalu-

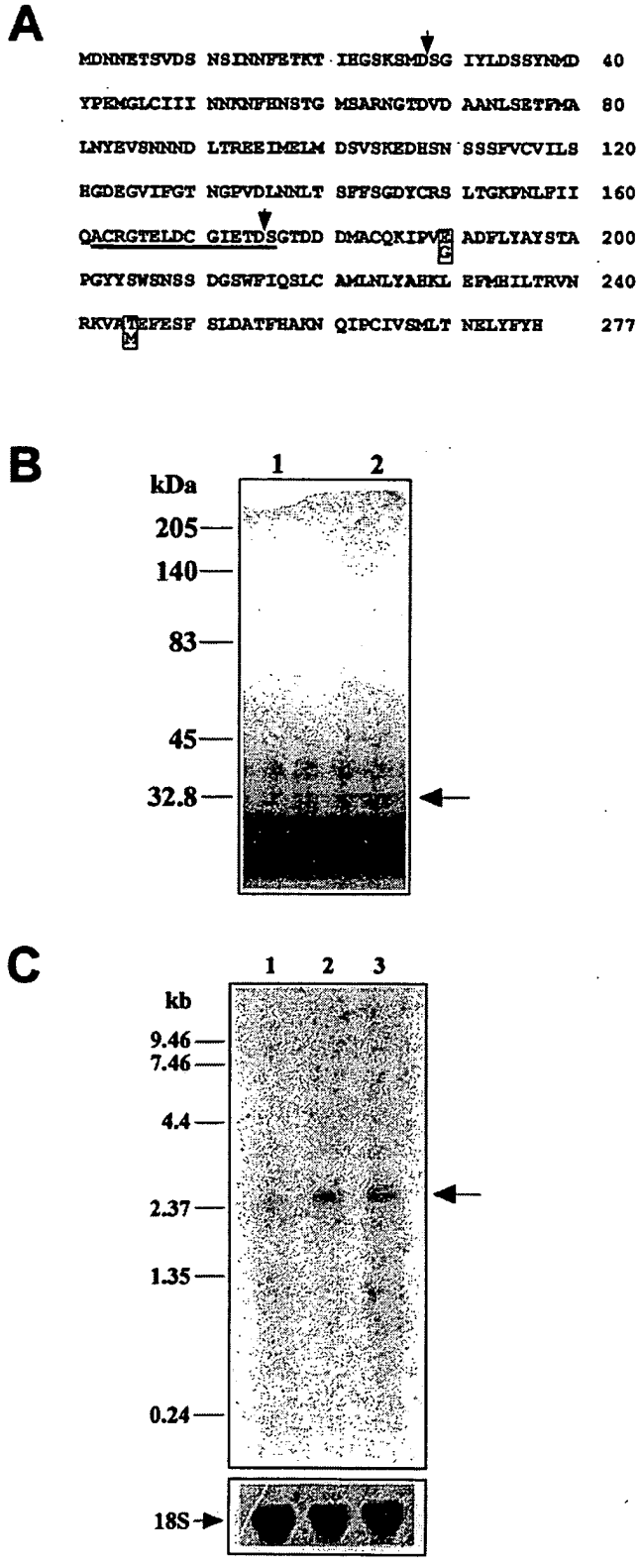

Figure 1. *A*, Deduced amino acid sequence of rat brain caspase-3 from the cDNA clone. This sequence shares 84.1% identity with the human cpp32β (Fernandes-Alnemri et al., 1994) and 99.3% with the rat brain IRP (Ni et al., 1997). The *boxes* mark the different amino acids between caspase-3 and the rat brain IRP. *Arrowheads* indicate the known proenzyme cleavage sites for caspase-3 (Asp28–Ser29 and Asp175–Ser176) that yield the p17 and p12 active forms. The peptide sequence used to raise the caspase-3 antibody is *underlined*. *B*, SDS-PAGE analysis of extract of *in vitro* translation assay product from caspase-3 cDNA. *Lane 1*, Negative control. cRNA was omitted from the assay. *Lane 2*, Translation product (*arrow*) from the caspase-3 cDNA. *C*, Northern blot analysis of caspase-3 mRNA in the hippocampus after sham operation (*lane 1*) or 8 hr (*lane 2*) or 24 hr (*lane 3*) after ischemia. Total RNA was isolated from the hippocampi (three brains per time point) and electrophoresed through a 1% agarose–formaldehyde gel (20 μg of RNA per *lane*). The only transcription species resulting from hybridizing with the caspase-3 cDNA probe is ~2.6–2.7 kb (*arrow*), consistent with the predicted molecular size of rat caspase-3 mRNA. *Bottom*, The same blot hybridized with the 18S RNA probe as a control for sample loading.

ated (see Results). For the measurement of caspase-3-like protease activity in peptide- or vehicle-infused brains, the brains were quickly removed 48 hr after ischemia and processed for the enzyme assay as described above.

For histological outcome experiments, rats were killed at 3 or 7 d after ischemia. Their brains were perfused and paraffin-imbedded, and 6 μm sections were cut. Coronal sections at the level of the dorsal hippocampus (anteroposterior, −4.0 mm from the bregma) were selected and stained with cresyl violet. Adjacent brain sections were processed for TUNEL staining using the method described above, except that the sections were pretreated with 1% H_2O_2 for 15 min to quench endogenous peroxidase before incubation in TdT buffer, and the biotin-16-dUTP tailing was detected using the horseradish-streptavidin-peroxidase method (Chen et al., 1997b). Sections were examined by two investigators who were blinded to the experimental conditions. Surviving hippocampal CA1 neurons (showing normal morphology by cresyl violet staining) and cells containing DNA fragmentation (TUNEL positive) were quantified with the assistance of a computerized scanning program (MCID; St. Catharine's).

To determine the potential role of the ICE-like protease in ischemic cell death, we subjected additional rats to ventricle infusion of the ICE inhibitor *N*-benzyloxycarbonyl-Tyr-Val-Ala-Asp(OMe)-CH_2F (Z-YVAD-FMK) (Enzyme Systems Products) before and after ischemia using the same protocol described for Z-DEVD-FMK. Rats received either Z-YVAD-FMK at 0.5 μg × 3 or 1.5 μg × 3 or the same volume of 0.04% DMSO (n = 6 per group). Three days after ischemia, the brains of these rats were processed for cresyl violet staining, TUNEL staining, and subsequent cell counting in the hippocampal CA1 sector.

Data analysis

All data are reported as mean ± SEM. Comparisons of caspase-3 mRNA expression, caspase-3 protein expression, caspase-3 activity, ICE-like activity, CA1 cell survival, or TUNEL-positive cells at different durations of reperfusion with or without inhibitor treatment versus sham controls were made using ANOVA and *post hoc* Fisher's PLSD tests. A level of $p < 0.05$ was considered statistically significant.

RESULTS

cDNA cloning of rat brain caspase-3

To characterize the expression pattern of the caspase-3 gene in the brain after ischemia, we cloned a cDNA containing the entire open reading frame of caspase-3 from the ischemic brain cDNA library. Sequence analysis revealed that this cDNA encodes an open reading frame of 277 amino acids (Fig. 1*A*). The deduced amino acid sequence had 84.1 and 92.1% identity to the published sequences of human and mouse cpp32β (Fernandes-Alnemri et al., 1994; Tewari et al., 1995), respectively. The positions of several predicted functional residues were also identical to those of human and mouse cpp32β, including the two aspartic acid residues (28 and 175) predicted to be the cleavage sites, the three residues required for substrate binding (Arg64, His108, and Arg207), and the QACRG motif responsible for binding of the protease to the aspartic acid residues in the substrate cleavage sites (Nicholson et al., 1995; Juan et al., 1996). After the comple-

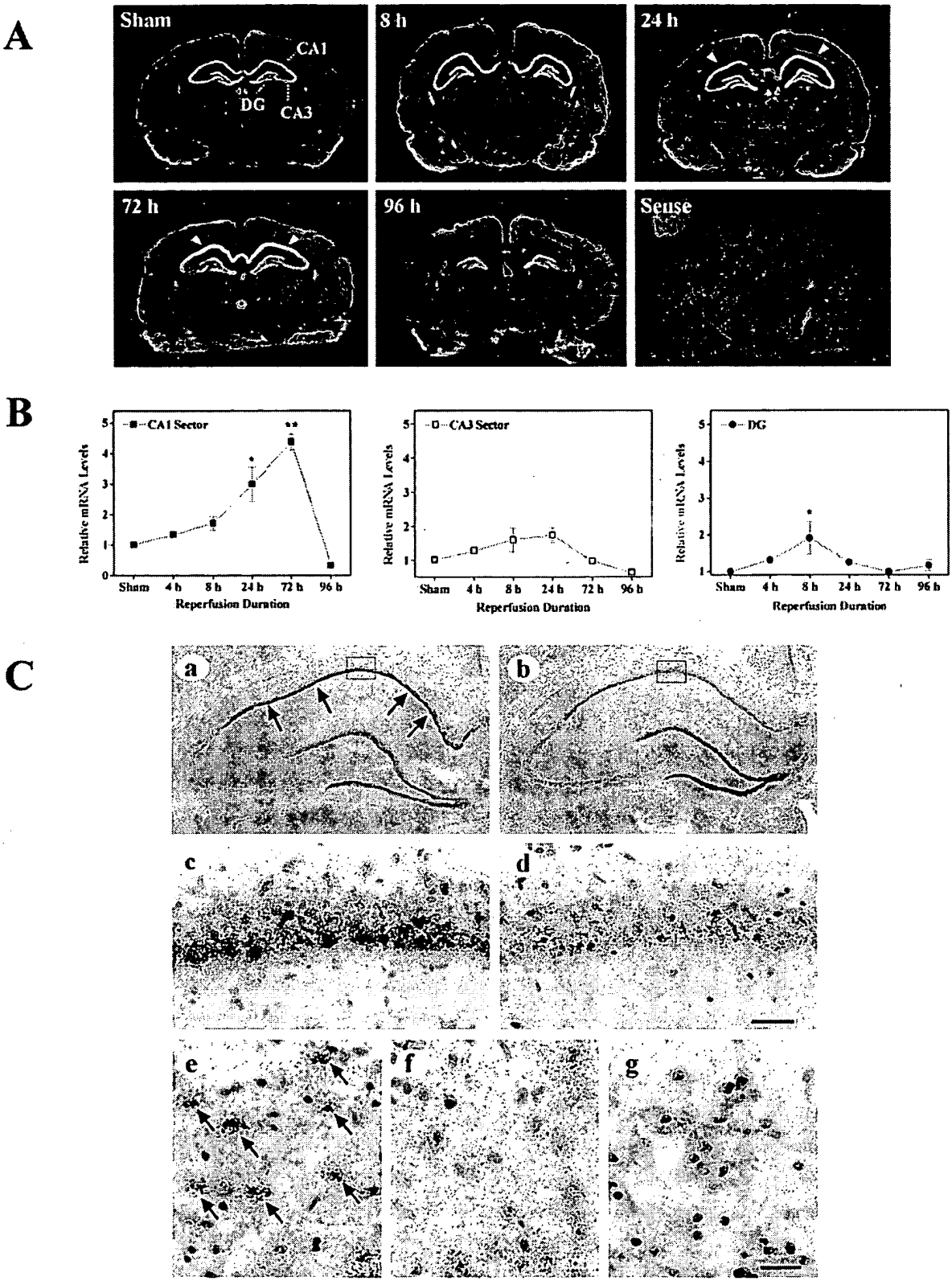

Figure 2. Caspase-3 *in situ* hybridization. *A*, Representative autoradiograms through the level of dorsal hippocampus in a sham-operated brain and in brains at 8, 24, 72, and 96 hr after 15 min of global ischemia. Caspase-3 mRNA is slightly increased in the dentate gyrus (*DG*) granule cell layer 8 hr after ischemia but markedly increased in the hippocampal *CA1* sector at 24 and 72 hr after ischemia (*arrowheads*). A section (24 hr after ischemia; *Sense*) hybridized with the sense cDNA probe results in only background signals. *B*, Relative caspase-3 mRNA changes in the (*Figure legend continues*)

tion of this work, two cDNA sequences encoding the rat caspase-3 homologs were published. One clone, obtained from a rat brain cDNA library and referred to as interleukin-converting enzyme-related protease (IRP) (Ni et al., 1997), was 99.3% identical to our amino acid sequence. The other clone (cpp32β), cloned from a rat colon cDNA library (Juan et al., 1996), was identical to our sequence at both nucleotide and amino acid levels. Thus, caspase-3 seems to be highly conserved across species and, within a species, across different organs.

Using the cloned cDNA as a template, we found that the *in vitro* transcription and translation assay produced a protein at ~32 kDa (Fig. 1*B*), the predicted size for caspase-3.

Evidence of caspase-3 gene induction after ischemia

Previous studies suggest that expression of caspase-3 mRNA and protein is retained in most adult tissues (Juan et al., 1996; Krajewska et al., 1997; Ni et al., 1997). In the present study, we have examined the expression of caspase-3 at both mRNA and protein levels in normal and ischemic brains, focusing on regions such as the hippocampus and caudate-putamen where cells are particularly vulnerable to transient global ischemia. Using Northern blot analysis, we detected caspase-3 mRNA (~2.6–2.7 kb) in all samples tested (Fig. 1*C*). Although caspase-3 mRNA was readily detected in the normal hippocampus, the levels were increased at 8 hr (1.8-fold) and 24 hr (2.3-fold) after 15 min of ischemia.

As shown in Figure 2*A–C*, the cellular distribution of the mRNA was examined further using *in situ* hybridization in nonischemic brains and in brains 4, 8, 24, 72, or 96 hr after ischemia (n = 3 per time point). Consistent with the findings in Northern blots, basal expression of caspase-3 mRNA was readily detectable in the whole hippocampal formation, including pyramidal neurons in the CA1–CA3 sectors and granule cells in the dentate gyrus (Fig. 2*A*). Low levels of basal expression were also present in medium-sized neurons in the caudate-putamen and thalamus and in large- and medium-sized neurons in the cortex. Eight hours after ischemia, significantly increased caspase-3 mRNA was first detected in the dentate granule cells (Fig. 2*B*). It subsided to near control levels in this region thereafter. In the CA1 sector, a slight increase in caspase-3 mRNA was first seen at 8 hr. The signals were markedly increased in this region at 24 and 72 hr after ischemia. Examination of emulsion-coated sections revealed that many neurons in the thalamus and dorsolateral putamen also showed increased caspase-3 mRNA signal at 24–72 hr after ischemia. In the cortex, however, only a few scattered shrunken neurons in layers III–V showed increased mRNA. In control experiments, sections hybridized with the sense cDNA probe showed a low-level background signal that was homogeneous throughout the brain sections (Fig. 2*A,C*).

Evidence of caspase-3 protein alteration after ischemia

Normally, caspase-3 protease is synthesized in cells as an inactive precursor (32 kDa). After activation, it is cleaved at the C terminal of two specific aspartic acid residues to form two mature subunits, p17 (17 kDa) and p12 (12 kDa) (Nicholson et al., 1995). Using the antibody against the deduced C-terminal sequence of the larger subunit (see Materials and Methods), we found that Western blots recognized both precursor and p17 but not p12 in brain protein extracts (Fig. 3). Normal nonischemic hippocampus and caudate-putamen showed fairly high levels of the caspase-3 precursor but none or very low levels of the p17 subunit. After ischemia, caspase-3 protein was increased in these regions (Fig. 3). In the hippocampus, the p17 subunit began to increase at 4 hr and markedly increased at 24–72 hr after ischemia. The levels of the precursor protein were also increased in this region at 8–72 hr. In the caudate-putamen, the p17 form was significantly increased and peaked at 8–24 hr after ischemia. The levels of the precursor protein, however, were unchanged after ischemia. The specificity of the caspase-3 immunoreactivity was confirmed in duplicate Western blots by preabsorbing the primary antibody with the specific caspase-3 peptide (5 μg/ml), which abolished the signals (data not shown). Neither the p17 nor the precursor protein was increased in the cortex 4–72 hr after ischemia (Fig. 3).

The cellular distribution of caspase-3 immunoreactivity in the brain was examined using immunohistochemistry. In contrast to the Western blot findings in normal nonischemic brains, neurons throughout most forebrain regions, including hippocampus, caudate-putamen, thalamus, and cortex, contained very weak or no caspase-3 immunoreactivity. As shown in Figure 4, basal caspase-3 immunoreactivity was exclusively present in the cell cytoplasm. Only very few scattered, shrunken neurons in the cortex and thalamus were highly caspase-3-immunoreactive and exhibited nuclear caspase-3 immunoreactivity. These results are consistent with recent observations in adult human brain tissues (Krajewska et al., 1997). Immunostaining in ischemic brains showed increased caspase-3 reactivity in several regions affected by global ischemia (Fig. 4*a–f*). In the hippocampal CA1 (but not CA3) neurons, increased caspase-3 immunoreactivity began to be detectable at 8 hr after ischemia and became maximal at 72 hr. At the earlier time points (8–24 hr), most cells in the CA1 sector had normal morphology, and the immunoreactivity was predominantly present in the cytosol. At 72 hr after ischemia, however, the majority of cells in this region (>90%) showed pyknotic changes (shrinkage of the cell body and condensation of the nucleus). The increased immunoreactivity was distributed throughout the entire cell, including the nucleus. The hippocampal dentate granule cell layers also showed mildly increased caspase-3 immunoreactivity at 8 hr but not at 24 or 72 hr after ischemia. In the dentate, immunoreactivity was diffusely distributed through the cell bodies. The other brain region showing a marked increase in cellular caspase-3 immunoreactivity after ischemia was the caudate-putamen. Many neurons in this region showed increased caspase-3 immunoreactivity 24–72 hr after ischemia. Many cells had pyknotic changes and showed increased immunoreactivity in the nucleus.

←

hippocampal CA1 sector, CA3 sector, and dentate gyrus at 4, 8, 24, 72, and 96 hr after ischemia versus sham controls (n = 3 per group), determined by optical density measurement on autoradiograms. Data are reported as mean ± SEM and represent fold changes in ischemic brains versus sham controls; * $p < 0.01$, and ** $p < 0.001$ versus sham controls (ANOVA and *post hoc* Fisher's PLSD tests). *C*, Representative emulsion-coated sections counterstained with cresyl violet from a brain 72 hr after ischemia (*a*, *c*, *e*) and a sham control brain (*b*, *d*, *f*). Caspase-3 mRNA is predominantly increased in the CA1 sector of ischemic hippocampus (*a*; *arrows*) compared with the control brain (*b*). Under a high-power field (400×), increased amounts of silver grains localize to CA1 pyramidal neurons (*c*) and to scattered neurons in the caudate-putamen (*e*; *arrows*) compared with the controls *d* and *f*, respectively. The *open squares* in *a* and *b* mark the regions from which the high-power views in *c* and *d* are taken, respectively. *g*, Sense control in caudate-putamen from the same ischemic brain. Scale bar, 30 μm.

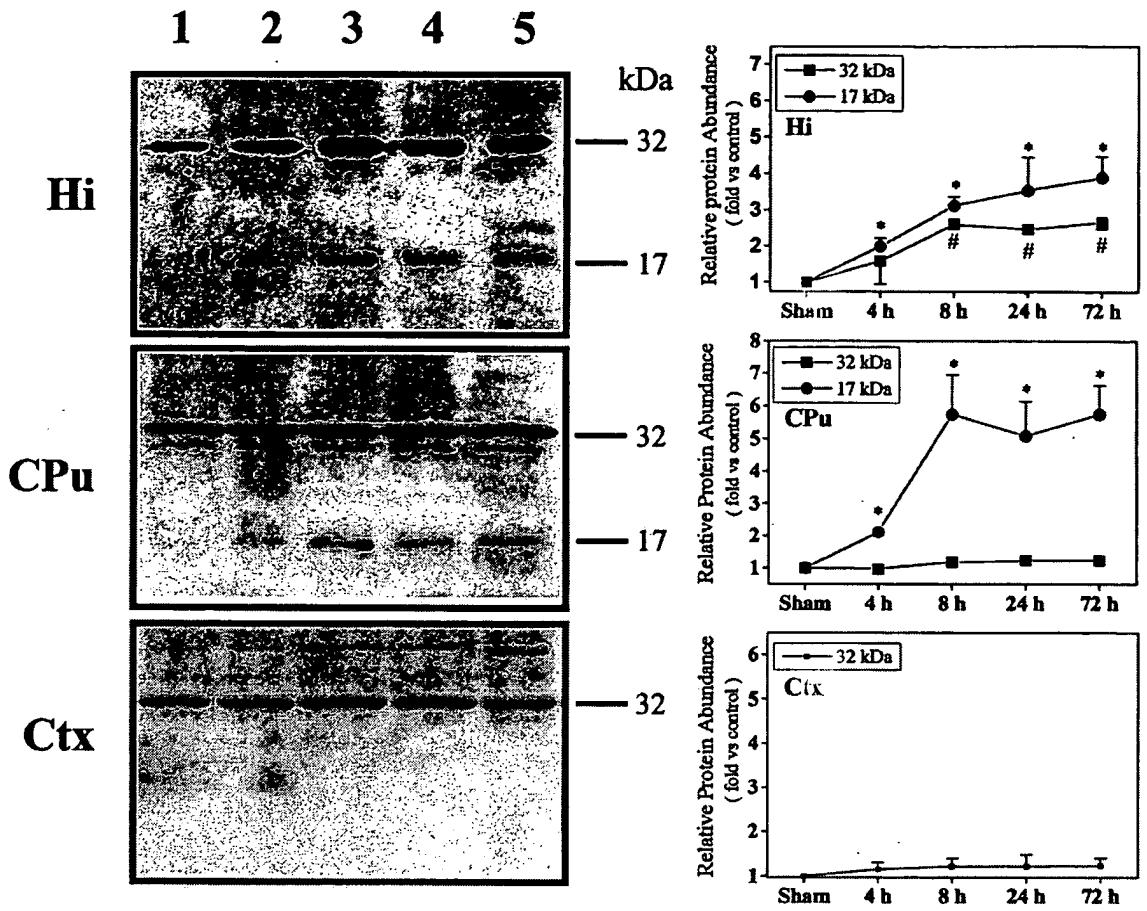

Figure 3. Western blot analysis of caspase-3 protein in the hippocampus (*Hi*), caudate-putamen (*CPu*), and cortex (*Ctx*) from brains after sham operation (*lane 1*) or 4 hr (*lane 2*), 8 hr (*lane 3*), 24 hr (*lane 4*), or 72 hr (*lane 5*) after ischemia. Immunoreactivity of both caspase-3 precursor protein (32 kDa) and its larger cleavage form (17 kDa) is increased in the hippocampus after ischemia. Immunoreactivity of the 17 kDa cleavage form is also increased in the caudate-putamen but not in the cortex. *Graphs*, Semiquantitative changes of caspase-3 protein after ischemia, determined by optical density (OD) measurements on Western blot autoradiograms (OD × area). Data are mean ± SEM (n = 4 per time point) and represent fold changes in ischemic brains versus sham controls; $^{*,\#}p < 0.05$ versus sham controls (ANOVA and *post hoc* Fisher's PLSD tests).

To address the potential role of caspase-3 protein induction in ischemic cell death, we performed double staining for caspase-3 protein and DNA fragmentation in brain sections obtained 24 and 72 hr after ischemia, when substantial cell death began to occur in the caudate-putamen and hippocampus, respectively. In the hippocampal CA1 sector, the majority of DNA-fragmented (TUNEL-positive) neurons showed increased nuclear caspase-3 immunoreactivity at 72 hr (Fig. 4*e–h*) and vice versa. In the caudate-putamen, especially the dorsolateral portion, many pyknotic cells showed colocalization of increased nuclear caspase-3 immunoreactivity and DNA fragmentation at 24 and 72 hr after ischemia (Fig. 4*f,h*). Colocalization of increased nuclear caspase-3 immunoreactivity and DNA fragmentation was also detected in a few scattered neurons in layers III and V of the cortex. In sham-operated control brains (n = 4), zero to three cells per section showed DNA fragmentation. No colocalization of DNA fragmentation and caspase-3 immunoreactivity was found in these brains.

TUNEL staining was also performed in adjacent brain sections using the horseradish-streptavidin-peroxidase method (Chen et al., 1997b), and positive versus negative cells in the hippocampus and caudate-putamen (CPu) were quantified. Twenty-four hours after ischemia, 31.5 ± 8.9% (mean ± SEM; n = 4) of the total number of cells in the CPu at the midcaudate level showed TUNEL-positive staining, whereas <0.5% of the CA1 neurons stained positively. At 72 hr after ischemia, however, 86.5 ± 4.3% of the neurons in the CA1 and 52.7 ± 11.4% of the cells in the CPu were TUNEL positive (mean ± SEM; n = 4 at each coronal level). In both regions, the majority of TUNEL-positive cells exhibited shrinkage of cytoplasm and nucleus and condensation of chromatin. Many cells in the CPu also showed formation of two or more dense masses around the nucleus suggestive of apoptotic bodies, as described by others (Li et al., 1995; Charriaut-Marlangue et al., 1996). Meanwhile, only a small portion of TUNEL-positive cells in the CA1 sector (<10% of the total number of positive cells), mainly in CA1a, exhibited significant morphological changes indicative of necrosis, such as diffused cytosol staining or loss of cellular structures (Charriaut-Marlangue et al., 1996). Thus, many but not all ischemic neurons in this model show morphological features of apoptotic cell death.

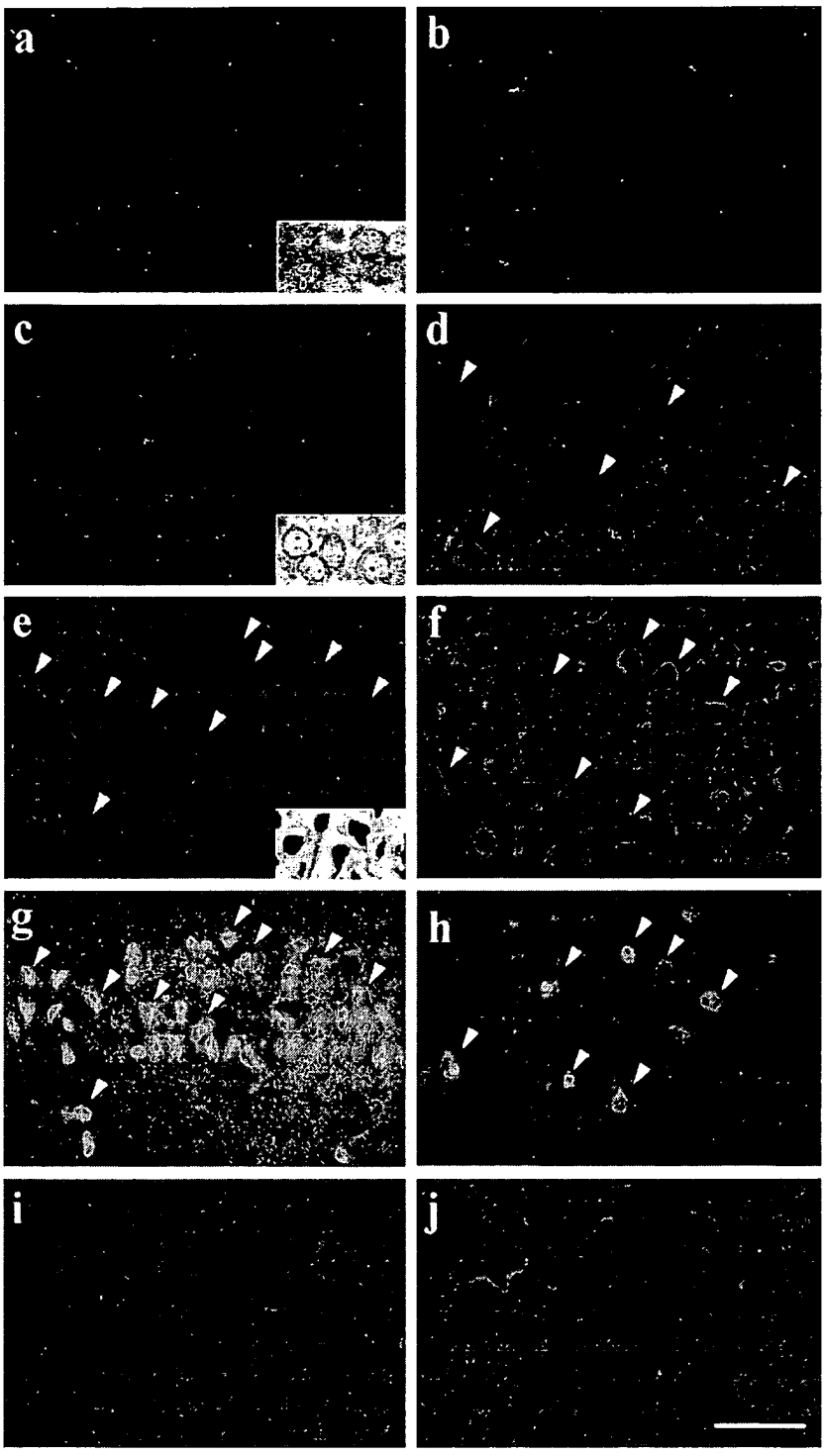

Figure 4. Immunofluorescent images of caspase-3 protein (*a–f, i, j*) and TUNEL labeling (*g, h*) in the hippocampal CA1 sector (*left*) and caudate-putamen (*right*) after ischemia. Compared with that in the control brain (*a, b*), caspase-3 immunofuorescence is increased in the cytoplasm of CA1 pyramidal neurons (*c*) and in scattered neurons in the caudate-putamen (*d*; *arrowheads*) at 24 hr after ischemia. Caspase-3 immunofluorescence is increased further in neurons in these regions with both cytosolic and nuclear localization at 72 hr after ischemia (*e, f*; *arrowheads* mark representative positive cells). Double-label (TUNEL) in the same sections shows a colocalization of DNA fragmentation (*g, h*; *arrowheads* mark representative positive cells) and increased caspase-3 immunofluorescence in most CA1 neurons (*e* vs *g*) and in many caudate neurons (*f* vs *h*) at 72 hr after ischemia. Note that TUNEL-positive neurons show a condensed and shrunken nucleus. Consecutive sections of *c* and *d* incubated with the primary antibody preabsorbed with the synthetic caspase-3 peptide show background fluorescence only (*i, j*). *Insets* in *a*, *c*, and *e* show representative cresyl violet staining in the CA1 sector. In keeping with the delayed manner of cell death in this model, CA1 neurons show normal morphology in control brain (*a*) and in the brain 24 hr after ischemia (*c*) but show pyknotic changes in the brain 72 hr after ischemia (*e*). Scale bar, 50 μm.

These observations are consistent with previous findings in similar animal models (Kihara et al., 1994; Nitatori et al., 1995; Chen et al., 1996).

Alteration of caspase-3- and ICE-like protease activities after ischemia

To assay for protease activity, we incubated cell lysates with Ac-DEVD-*p*NA as a substrate for caspase-3 and Ac-YVAD-*p*NA as a substrate for ICE and monitored release of *p*-nitroanilide. Lysates from normal nonischemic brains showed low levels of both ICE- and caspase-3-like peptide cleavage activities, which were blocked, but not completely, by saturated concentrations of the ICE inhibitor YVAD-CHO (5 μM) and the caspase-3 inhibitor DEVD-CHO (5 μM). The levels measured after inhibitor treatment were considered nonspecific background signals present in the lysates and were subsequently subtracted from all readings, as suggested previously (Enari et al., 1996). Lysates

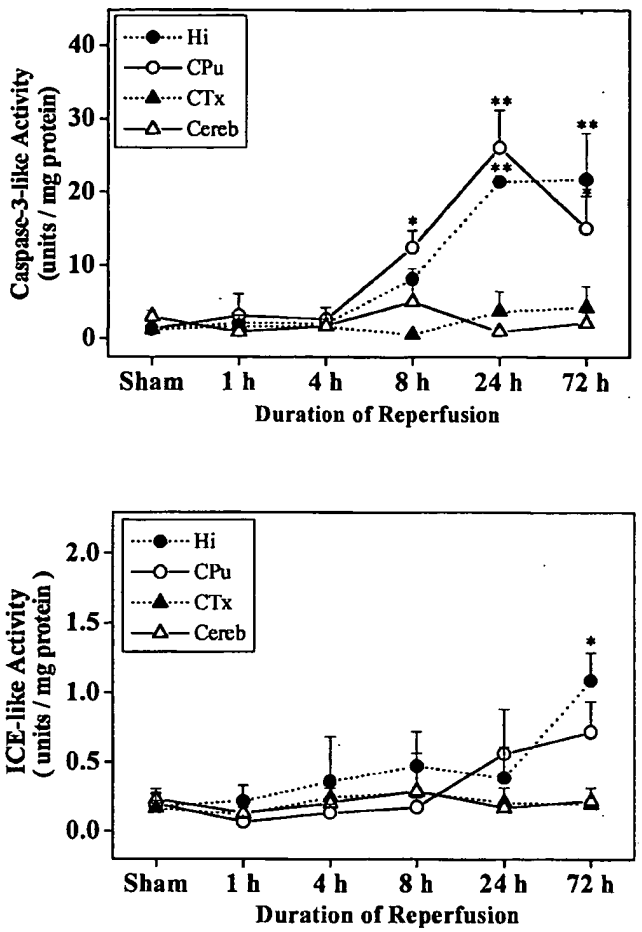

Figure 5. The caspase-3-like (*top*) and ICE-like protease activity (*bottom*) in cell extracts from the hippocampus (*Hi*), caudate-putamen (*CPu*), cortex (*CTx*), and cerebellum (*Cereb*) after sham operation or 1, 4, 8, 24, or 72 hr after ischemia. The protease activities are measured by determining the ability of cell extracts to cleave the colorimetric substrates Ac-DEVD-*p*NA (for caspase-3-like activity) and Ac-YVAD-*p*NA (for ICE-like activity). One unit of protease activity corresponds to the caspase-like activity that cleaves 1 pmol of *p*NA per minute at 37°C at saturating substrate concentrations. Data are presented as mean ± SEM (n = 4–5 per time point); $^{*}p < 0.05$, and $^{**}p < 0.01$ versus sham controls (ANOVA and *post hoc* Fisher's PLSD tests).

from the ischemic hippocampus and caudate-putamen exhibited an increase in Ac-DEVD-*p*NA peptide cleavage activity that was detectable at 8 hr and maximal at 24–72 hr after ischemia (Fig. 5, *top*). The increased caspase-3-like protease activity was completely inhibited by DEVD-CHO but was not affected by YVAD-CHO (data not shown). Moreover, lysates from ischemic hippocampus also exhibited a mild increase in Ac-YVAD-*p*NA cleavage activity that was detectable only at 72 hr after ischemia (Fig. 5, *bottom*). By contrast, there was no significant induction of ICE-like activity in the caudate-putamen at any time point tested. Neither caspase-3-like nor ICE-like protease activity was significantly increased in the cortical or cerebellum lysates at any time point after ischemia.

The finding that ischemia markedly induces Ac-DEVD-*p*NA cleaving activity in both the hippocampus and caudate-putamen prompted an analysis of poly(ADP-ribose) polymerase (PARP) cleavage and other potential caspase-3 cleavage substrates. Western blots using the C-2–10 monoclonal antibody detected increased cleavage of intact PARP (116 kDa) to the 85 kDa apoptosis-related cleavage fragment in ischemic brains (Fig. 6). The 85 kDa fragment was present at negligible levels in normal nonischemic brains. However, these levels were significantly increased after ischemia (24–72 hr after ischemia in the caudate-putamen and 72 hr after ischemia in the hippocampus). Duplicate Western blots performed using the primary antibody preabsorbed with the purified bovine PARP protein (10 μg/ml) showed a complete loss of signal at both 116 and 85 kDa (Fig. 6*a*), confirming the specificity of the immunoreactivity recognized by the PARP antibody. Moreover, PARP cleavage was not detected in the cortex 4–72 hr after ischemia (data not shown).

Considering that induced caspase-3 protease activity after ischemia may result in the cleavage of other substrates in the ischemic brain as well, we also analyzed two other proteins, the DNA-dependent protein kinase and actin. Both proteins have been found to be cleaved under certain apoptotic conditions in several cell systems (Casciola-Rosen et al., 1995; Mashima et al., 1995; Song et al., 1996; McConnell et al., 1997), however, no specific cleavage products from either of the two proteins were detected in cell extracts from the hippocampus or caudate-putamen with or without ischemic insults (data not shown).

Effects of inhibition of caspase-3-like protease activity after ischemia

To investigate the cell death regulatory role of caspase-3 in ischemic brain injury further, we infused Z-DEVD-FMK, an inhibitor of caspase-3, into the ventricles beginning 30 min before the induction of ischemia. DMSO was used as a vehicle. The doses of Z-DEVD-FMK used in the histological outcome studies were predetermined by a set of dose–response experiments, in which the ability of Z-DEVD-FMK to inhibit caspase-3-like protease activity in ischemic brains was examined.

Animals that received either no infusion, vehicle infusion, or peptide infusion all survived the experiments. At the highest dose (1.5 μg × 3), Z-DEVD-FMK did not cause any notable behavioral abnormalities in the animals or microscopic evidence of cell death in normal nonischemic brains, determined by cresyl violet staining 72 hr after infusion (n = 4). Brain and rectal temperatures were not altered by infusion of the peptide before, during, or after ischemia. The EEG reached isoelectricity within 10 sec of the induction of ischemia in all animals. In the brains of animals subjected to 15 min of ischemia followed by 48 hr of reperfusion, Z-DEVD-FMK infusion decreased Ac-DEVD-*p*NA peptide cleavage activity in the hippocampus in a dose-dependent manner (Fig. 7*aE*).

In histological outcome studies, Z-DEVD-FMK partially but significantly decreased neuronal death in the hippocampal CA1 sector at 72 hr after ischemia (Fig. 7*aA*,*aB*). The protective effect of Z-DEVD-FMK was detectable at higher doses, and the effect was more apparent in the hippocampus on the side ipsilateral rather than contralateral to the infusion. To test the efficacy of Z-DEVD-FMK administrated after ischemia, we infused the peptide into the ventricles beginning 2 hr after ischemia and followed with a second infusion 22 hr later (total dose, 4.5 μg). Post-treatment with Z-DEVD-FMK increased CA1 neuron survival in the hippocampus bilaterally 72 hr after ischemia, with an efficacy similar to that of pretreatment. To determine whether Z-DEVD-FMK promotes CA1 neuron survival after a longer period of reperfusion, we gave two additional groups of animals either vehicle or peptide pretreatment (1.5 μg × 3), and brain

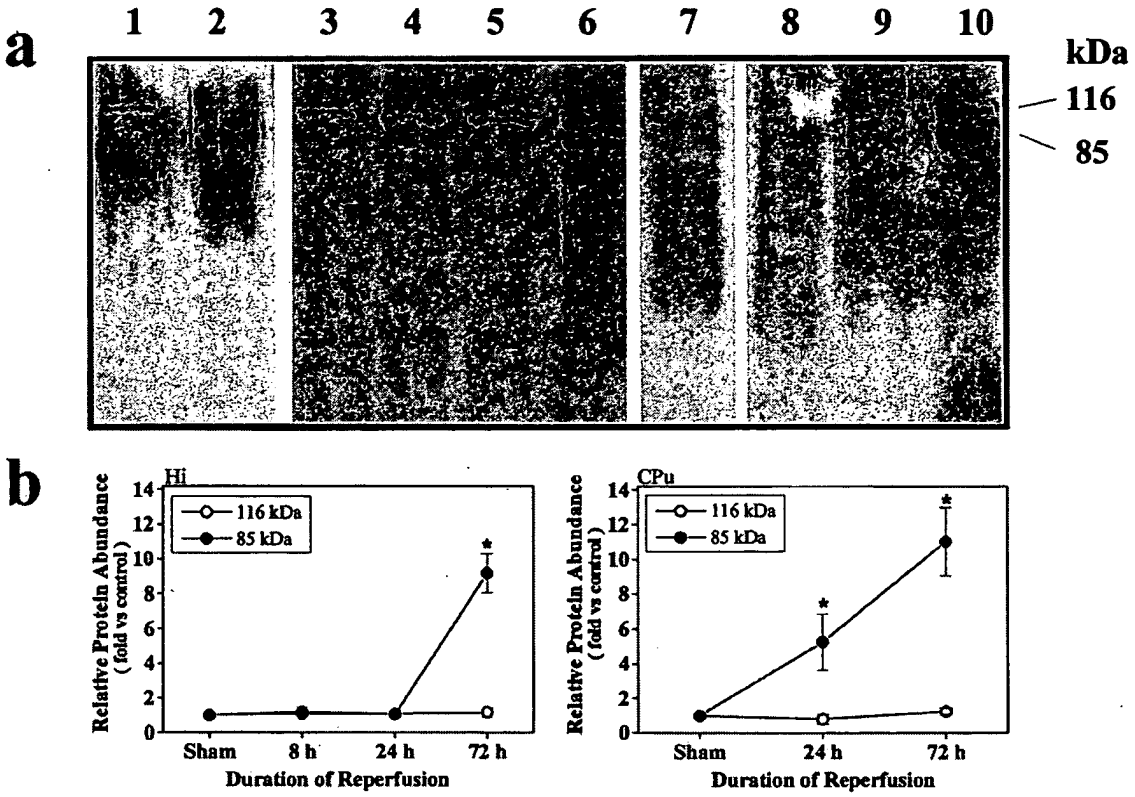

Figure 6. Western blot analysis of PARP in the hippocampus and caudate-putamen after ischemia. *a*, *Lanes 1–2*, Positive controls for intact PARP (116 kDa) and cleaved PARP (85 kDa) using cell extracts from uninduced human HL60 leukemia cells and HL60 induced to undergo apoptosis, respectively. *Lanes 3–6*, Cell extracts from the hippocampus after sham operation (*lane 3*) or 8 hr (*lane 4*), 24 hr (*lane 5*), or 72 hr (*lane 6*) after ischemia. Note that the 85 kDa form of PARP is increased at 72 hr. *Lane 7*, The same cell extract used in *lane 6* incubated with the primary antibody preabsorbed with purified bovine PARP protein (10 μg/ml). *Lanes 8–10*, Cell extracts from the caudate-putamen after sham operation (*lane 8*) or 24 hr (*lane 9*) or 72 hr (*lane 10*) after ischemia. Note that the 85 kDa form is increased in both ischemic samples. *b*, Semiquantitative analysis of relative PARP changes in the hippocampus (*Hi*) and caudate-putamen (*CPu*) after ischemia. Data are mean ± SEM ($n = 3$ per time point); $^*p < 0.05$ versus sham controls (ANOVA and *post hoc* Fisher's PLSD tests).

sections were obtained for analysis 7 d after ischemia. Z-DEVD-FMK significantly increased CA1 neuron survival, although to a lesser extent compared with the outcome at 3 d (Fig. 8). In all treatment paradigms tested (before or after treatment or a longer period of reperfusion), Z-DEVD-FMK significantly decreased the number of CA1 neurons that exhibited DNA fragmentation after ischemia (Fig. 7*aC*,*aD*). Representative micrographs showing histological outcome and DNA fragmentation after ischemia with or without peptide infusion are presented in Figure 8.

Because Z-DEVD-FMK, like all other commercially available caspase inhibitors, may act on multiple caspases instead of inhibiting caspase-3 only, another caspase inhibitor, Z-YVAD-FMK, which has a preferential action on ICE, was also tested in the present study. At both doses equivalent to the effective doses for Z-DEVD-FMK (0.5 μg × 3 and 1.5 μg × 3), Z-YVAD-FMK failed to show significant protection in CA1 neurons 3 d after ischemia (Fig. 7*b*).

DISCUSSION

A number of gene products may be modulators of neuronal apoptosis resulting from cerebral ischemia and related brain insults (for review, see Bredesen, 1995; Koistinaho and Hokfelt, 1997). In the present study, we demonstrate that caspase-3, a key member of the ICE protease family, was induced in neurons after transient global ischemia. Caspase-3 mRNA and protein were increased in the hippocampus and caudate-putamen, which are selectively vulnerable to ischemic injury. The caspase-3 precursor was proteolytically activated, and caspase-3-like protease activity was increased in these vulnerable brain regions before and coincidental with cell death. The specific caspase-3 protease substrate PARP was cleaved in these regions when substantial amounts of cell death occurred. Finally, inhibition of caspase-3-like protease activity using a tetrapeptide inhibitor significantly decreased neuronal death and DNA fragmentation in the hippocampal CA1 sector up to 7 d after ischemia. These results strongly support the hypothesis that the caspase-3 protease is an inducible cell death effector in the brain after ischemic injury.

Consistent with previous observations, caspase-3 mRNA and protein are present at low levels in the adult brain (Krajewska et al., 1997; Ni et al., 1997). After transient global ischemia, expression of both caspase-3 mRNA and protein was markedly increased in the brain. Although caspase-3 gene expression was transiently and modestly increased in less vulnerable cells such as dentate granule cells 8 hr after ischemia, a greater and prolonged increase, lasting up to 72 hr after ischemia, was found in selectively vulnerable CA1 and striatal neurons destined to die. These data confirm recent findings regarding cpp32 mRNA expression in a rat model of cardiac arrest (Gillardon et al., 1997). This pattern of expression is also similar to that of Bax, another

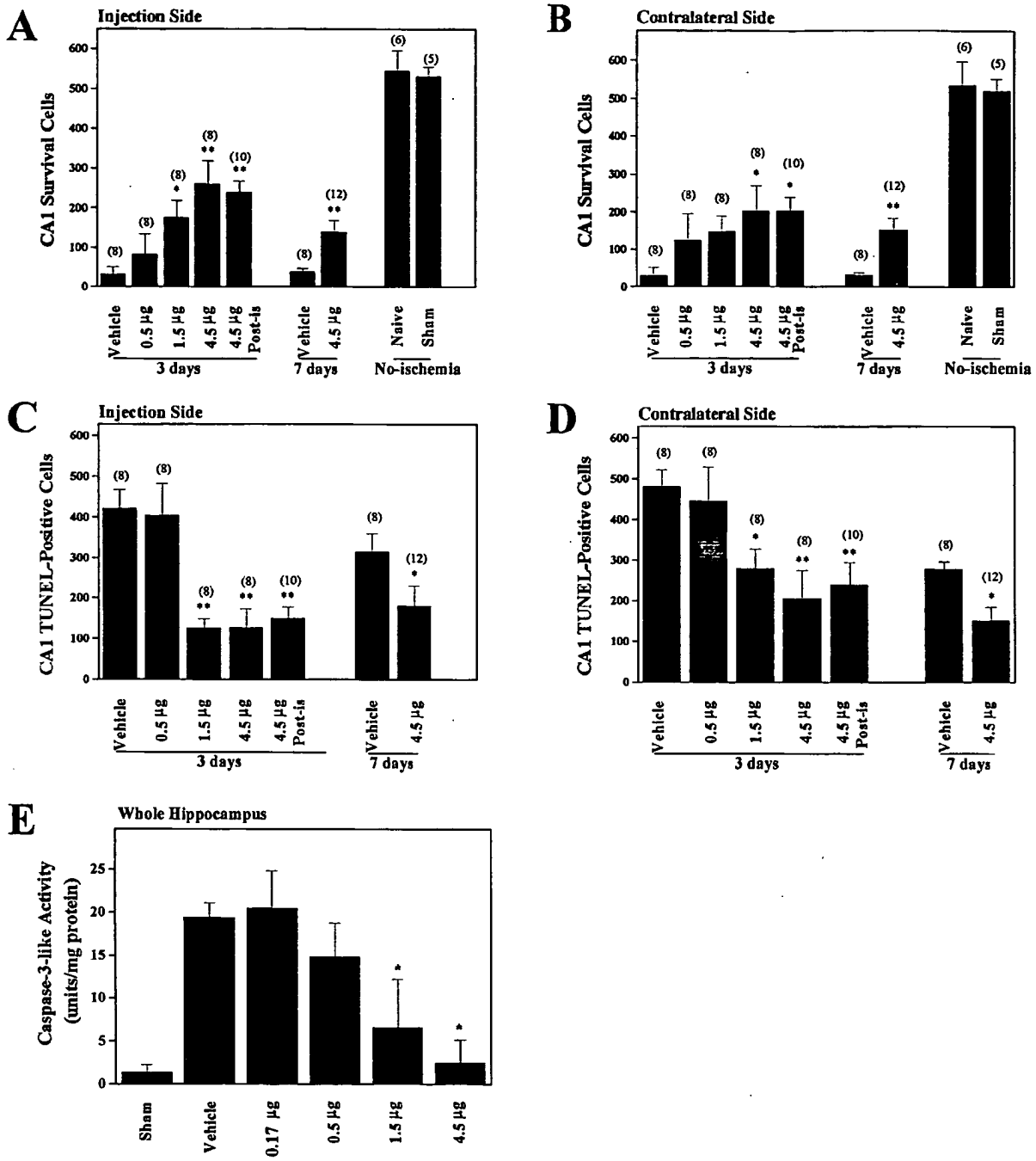

Figure 7. *a*, Quantitative analysis of effect of *in vivo* caspase-3 inhibitor treatment on hippocampal CA1 neuron survival (*A*, *B*), DNA fragmentation (*C*, *D*), and caspase-3-like activity after ischemia (*E*). *A*, *B*, Dose-dependent increase in CA1 neuron survival after ischemia by the caspase-3 inhibitor Z-DEVD-FMK, either injected before the induction of ischemia (vehicle or total dose of 0.5, 1.5, or 4.5 μg) or after ischemia (post-is with total dose of 4.5 μg). Cresyl violet staining and cell counting were performed either 3 or 7 d after ischemia. *C*, *D*, Dose-dependent decrease in the amount of cells with DNA fragmentation (TUNEL positive) in CA1 after ischemia by inhibiting caspase-3-like protease activity. No DNA fragmentation is present in CA1 in naive or sham-operated brains (data not shown). Sections through the same level of the dorsal hippocampus (bregma, −4.0 mm) are used for the analysis. Cell counting includes the entire CA1 sector at this level. *E*, Dose-dependent inhibition of caspase-3-like activity in the hippocampus by Z-DEVD-FMK. Vehicle ($n = 4$) or the peptide (total dose of 0.17, 0.5, 1.5, or 4.5 μg) was infused beginning 30 min before ischemia ($n = 5$ each dose). Caspase-3-like activity was measured in hippocampal cell extracts 48 hr after ischemia. All data are reported as mean ± SEM; * $p < 0.01$, and ** $p < 0.001$ (ANOVA and *post hoc* Fisher's PLSD tests). *b*, Quantitative analysis of effect of *in vivo* ICE inhibitor Z-YVAD-FMK treatment on hippocampal CA1 neuron survival (*A*) and DNA fragmentation (*B*) 3 d after ischemia. No significant protection by Z-YVAD-FMK was detected. *Injection Side*, The hemisphere receiving inhibitor infusion; *contralateral side*, the hemisphere receiving no infusion. The *number* in *parentheses* indicates the number of animals in that experimental group.

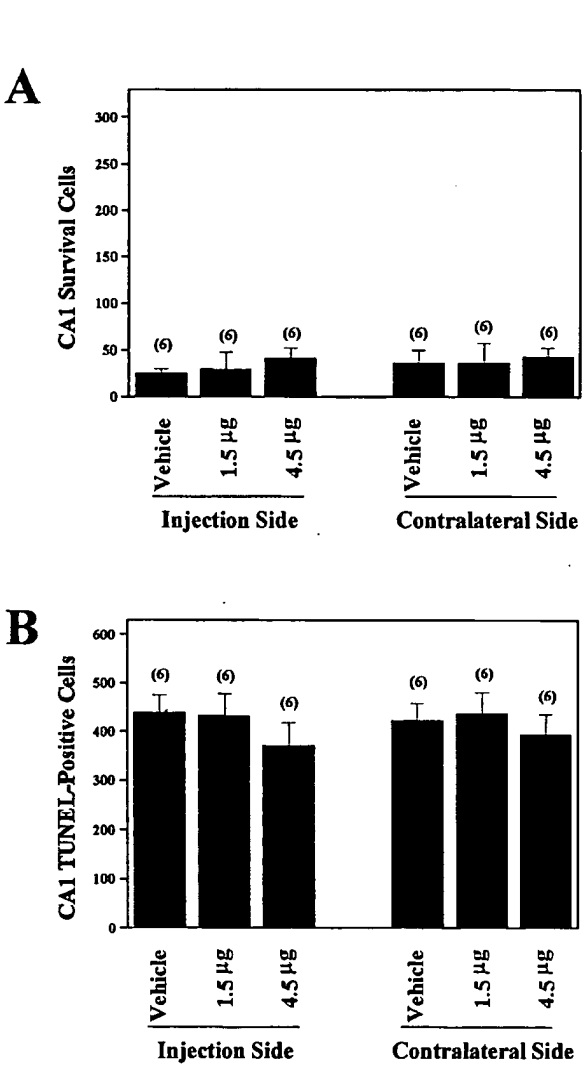

Figure 7b

proapoptotic gene studied in similar animal models of global ischemia (Krajewski et al., 1995; Chen et al., 1996). With rare exceptions, cells containing fragmented DNA also showed highly increased caspase-3 immunoreactivity (Fig. 4). Such colocalization of DNA fragmentation and elevated caspase-3 immunoreactivity was detected in a majority of neurons in the CA1 sector and many neurons in the CPu at 72 hr after ischemia. These results are consistent with a potential role for caspase-3 as a neuronal death effector in ischemic neurons.

As seen with other ICE family proteases, post-translational activation of caspase-3 requires proteolytic cleavage of the precursor protein, in this case into two subunits (p17 and p12), the larger of which contains the catalytic site (Fernandes-Alnemri et al., 1994). In the present study, an increase in p17 immunoreactivity began to be detectable in the hippocampus and CPu 4 hr after ischemia and peaked at 24–72 hr (Fig. 3). Nearly coincident with the time course of caspase-3 proteolytic cleavage after ischemia, caspase-3-like protease activity was selectively increased in the same brain regions, suggesting that proteolytic cleavage of caspase-3 may directly contribute to the increase in caspase-3 protease activity after ischemia. The increased caspase-3 protease activity was detected in brain regions in which cell death was eventually substantial (the hippocampus and CPu) but not in regions either less vulnerable to ischemia (the cortex) or unaffected by ischemia in this model (the cerebellum). Thus, we suggest that cell death after global ischemia is associated with post-translational activation as well as gene induction of caspase-3, with the proteolytic cleavage event slightly preceding gene upregulation. The mechanism by which the caspase-3 gene is induced and the caspase-3 precursor is cleaved after ischemia is unclear. Ischemia-induced glutamate release could be a trigger for caspase-3 gene induction (Du et al., 1997). A number of studies suggests that caspase-3 can be either autoactivated or activated by ICE or nedd2, Mch2a/CAP, Mch4, and Mch5 (Kumar et al., 1994; Tewari et al., 1995; Fernandes-Alnemri et al., 1996; Liu et al., 1996). It is possible that ischemia activates ICE or other upstream proteases, which lead to the activation of caspase-3. Subsequently, active caspase-3 and overexpression of caspase-3 could contribute to autoactivation of this enzyme. Accordingly, we speculated that ICE might be induced or activated during earlier reperfusion periods after ischemia. However, we could not detect increased ICE mRNA expression in the brain 0.5–72 hr after ischemia (data not shown). Moreover, ICE-like protease activity was only modestly increased in the hippocampus and only after 72 hr of reperfusion and was not increased in the CPu (Fig. 5). The ICE inhibitor Z-YVAD-FMK failed to provide significant protection in CA1 (Fig. 7*b*). These data do not support ICE as the main trigger responsible for the activation of caspase-3 and subsequent neuronal death after global ischemia.

The regional and temporal profile of caspase-3 induction after ischemia supports a role of this enzyme in ischemic neuronal death; however, a causal relationship between these two events cannot be deduced from the expression data alone. Consequently, we have studied the effect of inhibiting caspase-3 activity on cell survival after ischemia. The tetrapeptide inhibitor Z-DEVD-FMK significantly reduced caspase-3-like protease activity in ischemic hippocampus and significantly decreased CA1 cell loss, with a similar efficacy whether given before or after ischemia. The protective effect of Z-DEVD-FMK on CA1 neuron survival is unlikely to be caused by its effects on brain temperature or ischemia induction (as judged by EEG measurement). However, we must interpret these data with great caution. Because the tetrapeptide inhibitor was designed based on the PARP cleavage site (Lazebnik et al., 1994), caspase-3 may not be the sole caspase inhibited by Z-DEVD-FMK. Several caspase-3-related proteases including Mch2a and Mch3a are also capable of cleaving PARP or the *N*-acetyl-Asp-Glu-Val-Asp-(7-amino-4-methylcoumarin) (DEVD-AMC) substrate reminiscent of the PARP cleavage site (Fernandes-Alnemri et al., 1995). Ich-2/Tx and Ich-1/nedd2 can cleave PARP, but this requires very high enzyme–substrate ratios (for review, see Zhivotovsky et al., 1996). Thus, we cannot exclude the possibility that the protective effect achieved by the caspase-3 inhibitor is via the inhibition of other caspase-3-like enzymes. To address this issue, it will be helpful to compare ischemic injury in naive and caspase-3-deficient (knock-out) animals. Nevertheless, the fact that the caspase-3 gene is induced and its enzymatic activity is increased after ischemia provides strong evidence to link ischemic cell death to the activation of caspase-3.

Caspase-3 may mediate ischemic cell death via several mechanisms. Mature caspase-3 can cleave specific cellular proteins. Several proteins have been suggested as potential targets for caspase-3 during apoptosis. The best-characterized death sub-

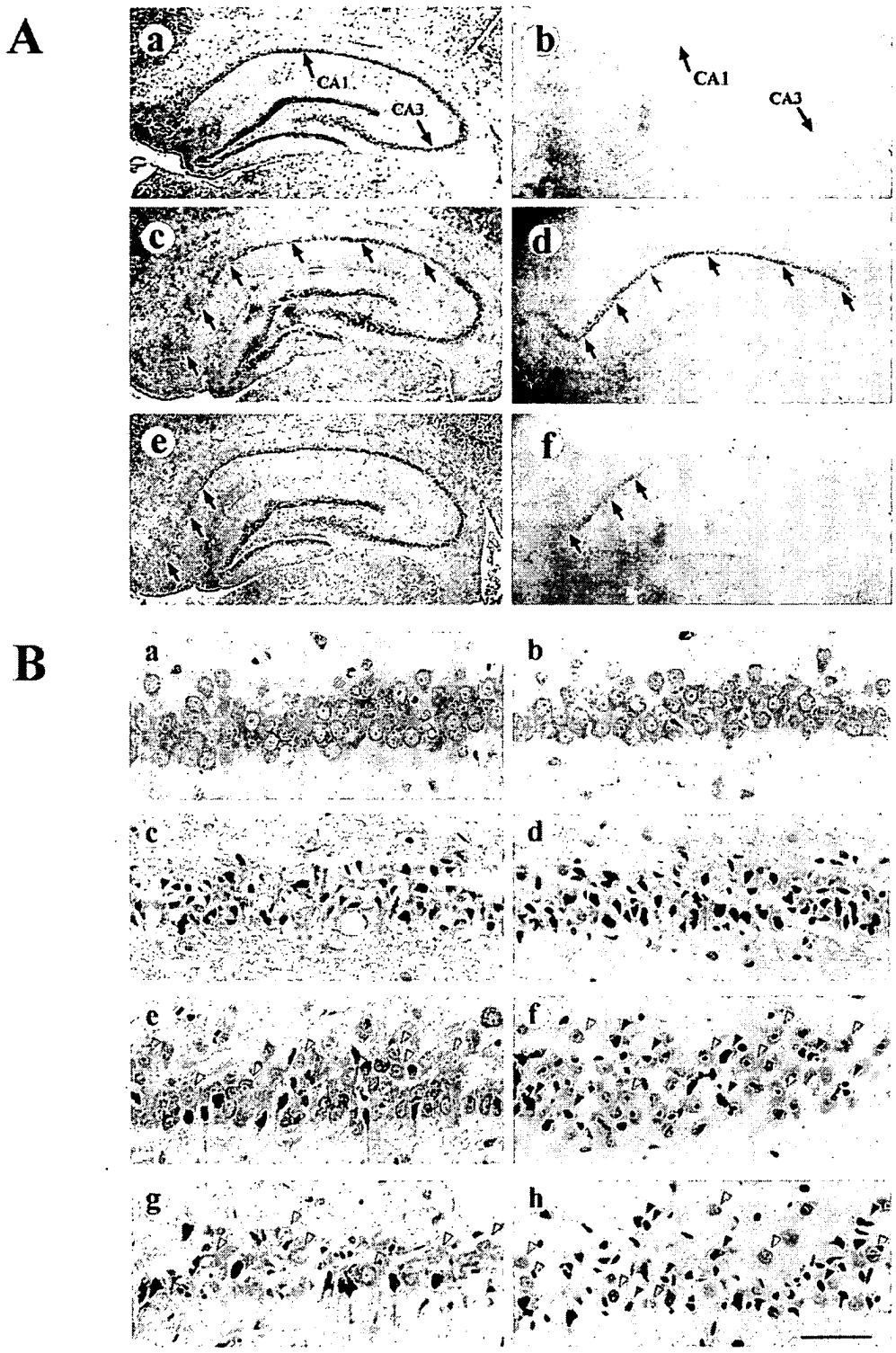

Figure 8. Effect of *in vivo* caspase-3 inhibition on CA1 neuron survival and DNA fragmentation. *A*, Low-power fields (40×) showing representative cresyl violet staining (*a*, *c*, *e*) and TUNEL (*b*, *d*, *f*) in the hippocampus 3 d after sham operation (*a*, *b*), after ischemia plus vehicle infusion (*c*, *d*), or after ischemia plus caspase-3 inhibitor infusion (total dose, 4.5 μg; *e*, *f*). *Arrows* in *c* and *e* mark cell death in the CA1 sector. *Arrows* in *d* and *f* mark DNA-fragmented (TUNEL-positive) cells in the CA1 sector. *B*, High-power fields (400×) showing representative cresyl violet staining (*a*, *c*, *e*, *g*) and TUNEL counterstained with cresyl violet (*b*, *d*, *f*, *h*) in the CA1 sector. Three days after sham operation (*a*, *b*), no cell death or DNA fragmentation is present in CA1; 3 d after ischemia plus vehicle infusion (*c*, *d*), the majority of CA1 neurons show pyknotic changes (*c*) and TUNEL labeling (*d*); 3 d after ischemia plus caspase-3 inhibitor infusion (*e*, *f*), many neurons show normal morphology (*yellow arrowheads*), and decreased amounts of neurons show pyknotic changes (*e*) or TUNEL labeling (*red arrowheads*; *f*); and 7 d after ischemia plus inhibitor infusion (*g*, *h*), both survival neurons (*yellow arrowheads*) and TUNEL-positive cells (*red arrowheads*; *h*) are present in the CA1. Scale bar, 50 μm.

strate is PARP, an enzyme involved in DNA repair and maintenance of genome integrity (Althaus and Richter, 1987; Satoh and Lindahl, 1992). Under many apoptotic conditions, PARP is cleaved by caspase-3 to generate the characteristic 85 and 24 kDa fragments (Kaufmann et al., 1993; Fernandes-Alnemri et al., 1995; Nicholson et al., 1995). Other proteins thought to be targets for caspase-3 include DNA-dependent protein kinase (DNA-PK) (Casciola-Rosen et al., 1995; Han et al., 1996), protein kinase C (Hugunin et al., 1996), the transcription factors SREBPs (Wang et al., 1996), and actin (Mashima et al., 1997; Song et al., 1997). In the present study, PARP was partially cleaved to its 85 kDa fragment in vulnerable brain regions after ischemia. No degradation of DNA-PK or actin was detected. Thus, PARP may be a specific substrate for caspase-3 during ischemic cell death, although whether degradation of PARP is an event leading to ischemic cell death is unclear. First, as shown in this study, cleavage of PARP is a late event after ischemia. Second, PARP is a nuclear protein, and although caspase-3 may be present in the nucleus of degenerated neurons (Fig. 4), there is no direct evidence that mature caspase-3 is relocated to the nucleus from the cytosol before cell death. Third, under certain circumstances such as focal cerebral ischemia and excitotoxicity, gene disruption or pharmacological inhibition of PARP improves neuronal survival (Eliasson et al., 1997; Endres et al., 1997). Accordingly, cleavage of PARP may not be responsible for neuronal death after ischemia. Instead, PARP degradation may reflect the overall cellular destruction in the final stages of the apoptotic cascade. Another potential mechanism via which caspase-3 might effect cell death after ischemia is by activating other caspases such as Mch2a and Mch6 involved in the apoptotic cascade (Srinivasula et al., 1996). Finally, caspase-3 may promote cell death via the activation of caspase-activated deoxyribonuclease (CAD), a key DNA-cleavage enzyme responsible for DNA fragmentation during apoptosis. Enari et al. (1998) recently suggest that caspase-3 activates CAD by cleaving and releasing the CAD inhibitory protein that normally binds CAD. Future work identifying specific cellular substrates for caspase-3 at early stages of cell death would greatly enhance our understanding of the role of this death protease in ischemic brain injury.

In summary, the present study provides evidence that the caspase-3 gene is induced and its protein product is activated in selectively vulnerable brain regions after ischemia. Caspase-3 activation precedes and its regional distribution correlates with delayed cell death. The caspase-3 inhibitor decreases protease activity in the hippocampus and increases cell survival in this region after ischemia. The results strongly support a cell death-effector role for caspase-3-protease in ischemic brain injury. Future development of highly specific methods to inhibit caspase-3-like protease activity or its consequences may have therapeutic significance in the treatment of stroke and related neurological disorders.

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Set     Items   Description
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S2   14442966   HUMAN OR MOUSE OR DROSOPHILA OR YEAST
S3        192   S1 AND S2
S4    1351035   CHIMER? OR CONJUGAT? OR HETEROLOGOUS OR COUPLED
S5          1   S3 AND S4
S6        121   S3 AND PY<=2001
S7        108   S6 AND PY<2001
S8          1   CYCLIN(W)DEPENDENT(W)KINASE(W)REGULAT?(5N)SUBUNIT
? s cyclin(w)dependent(5n)kinase
          62059  CYCLIN
        1913890  DEPENDENT
         721565  KINASE
     S9   25728  CYCLIN(W)DEPENDENT(5N)KINASE
? s s6 and s9
            121  S6
          25728  S9
    S10      37  S6 AND S9
? rd
>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
    S11      19  RD (unique items)
? t s11/3,k,ab/1-19

 11/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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14335498   PMID: 10323869
   Cyclin-dependent kinase and Cks/Suc1 interact with the
proteasome in yeast to control proteolysis of M-phase targets.
  Kaiser  P;  Moncollin V; Clarke D J; Watson M H; Bertolaet B L; Reed S I;
Bailly E
  The Scripps Research Institute, MB7, La Jolla, California 92037, USA.
  Genes  &  development (UNITED  STATES)  May 1 1999,  13  (9)
 p1190-202,  ISSN 0890-9369  Journal Code: 8711660
  Contract/Grant No.: GM38528; GM; NIGMS
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  Cell cycle-specific proteolysis is critical for proper execution of
mitosis in all eukaryotes. Ubiquitination and subsequent proteolysis of the
mitotic regulators Clb2 and Pds1 depend on the cyclosome/APC and the 26S
proteasome. We report here that components of the cell cycle machinery in
yeast, specifically the cell cycle regulatory cyclin-
dependent kinase Cdc28 and a conserved associated protein
Cks1 /Suc1, interact genetically, physically, and functionally with
components of the 26S proteasome. A mutation in Cdc28 (cdc28-1N) that
interferes with Cks1 binding, or inactivation of Cks1 itself,
confers stabilization of Clb2, the principal mitotic B-type cyclin in
budding yeast. Surprisingly, Clb2-ubiquitination in vivo and in vitro
is not affected by mutations in cks1, indicating that Cks1 is
not essential for cyclosome/APC activity. However, mutant Cks1
proteins no longer physically interact with the proteasome, suggesting that
Cks1 is required for some aspect of proteasome function during
M-phase-specific proteolysis. We further provide evidence that Cks1
function is required for degradation of the anaphase inhibitor Pds1.
Stabilization of Pds1 is partially responsible for the metaphase arrest
phenotype of cks1 mutants because deletion of PDS1 partially relieves
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the metaphase block in these mutants.

Cyclin-dependent kinase and Cks/Suc1 interact with the proteasome in **yeast** to control proteolysis of M-phase targets.
May 1 **1999**,
... and the 26S proteasome. We report here that components of the cell cycle machinery in **yeast**, specifically the cell cycle regulatory **cyclin-dependent kinase** Cdc28 and a conserved associated protein **Cks1**/Suc1, interact genetically, physically, and functionally with components of the 26S proteasome. A mutation in Cdc28 (cdc28-1N) that interferes with **Cks1** binding, or inactivation of **Cks1** itself, confers stabilization of Clb2, the principal mitotic B-type cyclin in budding **yeast**. Surprisingly, Clb2-ubiquitination in vivo and in vitro is not affected by mutations in **cks1**, indicating that **Cks1** is not essential for cyclosome/APC activity. However, mutant **Cks1** proteins no longer physically interact with the proteasome, suggesting that **Cks1** is required for some aspect of proteasome function during M-phase-specific proteolysis. We further provide evidence that **Cks1** function is required for degradation of the anaphase inhibitor Pds1. Stabilization of Pds1 is partially responsible for the metaphase arrest phenotype of **cks1** mutants because deletion of PDS1 partially relieves the metaphase block in these mutants.
; Base Sequence; CDC28 Protein Kinase, S cerevisiae--genetics--GE; CDC28 Protein **Kinase**, S cerevisiae--metabolism--ME; **Cyclin-Dependent** Kinases--genetics--GE; Cyclins--genetics--GE; Cyclins --metabolism--ME; Cysteine Endopeptidases--genetics--GE; DNA Primers...
Chemical Name: **CKS1** protein, S cerevisiae; CLB2 protein, **yeast**; Cell Cycle Proteins; Cyclin B; Cyclins; DNA Primers; Fungal Proteins; Multienzyme Complexes; Nuclear Proteins; PDS1...

...S cerevisiae; Saccharomyces cerevisiae Proteins; Schizosaccharomyces pombe Proteins; Suc1 protein, S pombe; Ubiquitins; CDC28 Protein **Kinase**, S cerevisiae; **Cyclin-Dependent** Kinases; Endopeptidases; Cysteine Endopeptidases; multicatalytic endopeptidase complex; Ligases; Ubiquitin-Protein Ligase Complexes; anaphase-promoting complex

11/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

14279103 PMID: 10100639
Mutational analysis of two Arabidopsis thaliana cyclin-dependent kinases in fission **yeast**.
Porceddu A; De Veylder L; Hayles J; Van Montagu M; Inze D; Mironov V; Porceddua A; De Veyldera L
Departement Genetica, Vlaams Interuniversitair Instituut voor Biotechnologie, Universiteit Gent, Belgium.
FEBS letters (NETHERLANDS) Mar 5 **1999**, 446 (1) p182-8, ISSN 0014-5793 Journal Code: 0155157
Erratum in FEBS Lett 1999 Jul 2;454(1-2) 172; Erratum in Note Porceddua A[corrected to Porceddu A];De Veyldera L[corrected to De Veylder L]
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have analyzed five mutant alleles of two cyclin-dependent kinases from Arabidopsis thaliana, CDC2aAt and CDC2bAt, in Schizosaccharomyces pombe. Two of the five mutant alleles produced similar phenotypes for both cyclin-dependent kinases. The other three mutants caused phenotypes dependent on the particular **cyclin-dependent kinase**. Of all the mutant alleles, only two were found to possess a detectable kinase activity. Our mutational analysis lends further support for CDC2aAt being

the true orthologue of the **yeast** cdc2. CDC2bAt, even though quite divergent from S. pombe cdc2, still retains the ability to interact with at least some essential cell cycle regulators, suggesting some functional homology with the **yeast** protein. Additionally, we demonstrated that the three amino acid deletion in the DL50 mutants results in the loss of the ability to interact with the suc1/**CKS1** proteins.

Mutational analysis of two Arabidopsis thaliana cyclin-dependent kinases in fission **yeast**.
Mar 5 **1999**,
... for both cyclin-dependent kinases. The other three mutants caused phenotypes dependent on the particular **cyclin-dependent kinase** . Of all the mutant alleles, only two were found to possess a detectable kinase activity. Our mutational analysis lends further support for CDC2aAt being the true orthologue of the **yeast** cdc2. CDC2bAt, even though quite divergent from S. pombe cdc2, still retains the ability to interact with at least some essential cell cycle regulators, suggesting some functional homology with the **yeast** protein. Additionally, we demonstrated that the three amino acid deletion in the DL50 mutants results in the loss of the ability to interact with the suc1/**CKS1** proteins.

11/3,K,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13860918 PMID: 9553063
The crk3 gene of Leishmania mexicana encodes a stage-regulated cdc2-related histone H1 kinase that associates with p12.
Grant K M; Hassan P; Anderson J S; Mottram J C
Wellcome Unit of Molecular Parasitology, University of Glasgow, The Anderson College, Glasgow G11 6NU, Scotland, United Kingdom.
Journal of biological chemistry (UNITED STATES) Apr 24 **1998**, 273 (17) p10153-9, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A cdc2-related protein kinase gene, crk3, has been isolated from the parasitic protozoan Leishmania mexicana. Data presented here suggests that crk3 is a good candidate to be the leishmanial cdc2 homologue but that the parasite protein has some characteristics which distinguish it from mammalian cdc2. crk3 is predicted to encode a 35.6-kDa protein with 54% sequence identity with the **human cyclin-dependent kinase** cdc2 and 78% identity with the Trypanosoma brucei CRK3. The trypanosomatid CRK3 proteins have an unusual, poorly conserved 19-amino acid N-terminal extension not present in **human** cdc2. crk3 is single copy, and there is 5-fold higher mRNA in the replicative promastigote life-cycle stage than in the non-dividing metacyclic form or mammalian amastigote form. A leishmanial suc-binding cdc2-related kinase (SBCRK) histone H1 kinase, has previously been described which binds the **yeast** protein, p13(suc1), and that has stage-regulated activity (Mottram J. C., Kinnaird, J., Shiels, B. R., Tait, A., and Barry, J. D. (1993) J. Biol. Chem. 268, 21044-21051). CRK3 from cell extracts of the three life-cycle stages was found to bind p13(suc1) and the leishmanial homologue p12(**cks1**). CRK3 fused with six histidines at the C terminus was expressed in L. mexicana and shown to have SBCRK histone H1 kinase activity. Depletion of histidine-tagged CRK3 from L. mexicana cell extracts, by Ni-nitrilotriacetic acid agarose selection, reduced histone H1 kinase activity binding to p13(suc1). These data imply that crk3 encodes the kinase subunit of SBCRK. SBCRK and histidine-tagged CRK3 activities were inhibited by the purine analogue olomoucine with an IC50 of 28 and 42 microM, respectively, 5-6-fold higher than **human** p34(cdc2)/cyclinB.

Apr 24 **1998**,
... is predicted to encode a 35.6-kDa protein with 54% sequence identity with the **human cyclin-dependent kinase** cdc2 and 78% identity with the Trypanosoma brucei CRK3. The trypanosomatid CRK3 proteins have an unusual, poorly conserved 19-amino acid N-terminal extension not present in **human** cdc2. crk3 is single copy, and there is 5-fold higher mRNA in the replicative...

... binding cdc2-related kinase (SBCRK) histone H1 kinase, has previously been described which binds the **yeast** protein, p13(suc1), and that has stage-regulated activity (Mottram J. C., Kinnaird, J., Shiels...

... three life-cycle stages was found to bind p13(suc1) and the leishmanial homologue p12(**cks1**). CRK3 fused with six histidines at the C terminus was expressed in L. mexicana and...

... olomoucine with an IC50 of 28 and 42 microM, respectively, 5-6-fold higher than **human** p34(cdc2)/cyclinB.
Tags: **Human**;
Chemical Name: Enzyme Inhibitors; Protozoan Proteins; Purines; Recombinant Proteins; olomoucine; p12 **cks1** protein, Leishmania mexicana; CDC2 Protein Kinase

11/3,K,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13135380 PMID: 8805536
Crystal structure of the **yeast** cell-cycle control protein, p13suc1, in a strand-exchanged dimer.
Khazanovich N; Bateman K; Chernaia M; Michalak M; James M
Medical Research Council Group in Protein Structure and Function, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.
Structure (London, England) (ENGLAND) Mar 15 **1996**, 4 (3) p299-309, ISSN 0969-2126 Journal Code: 9418985
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND. p13(suc1) from fission **yeast** is a member of the CDC28 kinase specific (CKS) class of cell-cycle control proteins, that includes **CKS1** from budding **yeast** and the **human** homologues CksHs1 and CksHs2. p13(suc1) participates in the regulation of p34(cdc2), a **cyclin-dependent kinase** controlling the G1-S and the G2-M transitions of the cell cycle. The CKS proteins are believed to exert their regulatory activity by binding to the kinase, in which case their function may be governed by their conformation or oligomerization state. Previously determined X-ray structures of p13(suc1), CksHs1 and CksHs2 show that these proteins share a common fold but adopt different oligomeric states. Monomeric forms of p13(suc1) and CksHs1 have been solved. In addition, CksHs2 and p13(suc1) have been observed by X-ray crystallography in assemblies of strand-exchanged dimers. Analysis of various assemblies of the CKS proteins, as found in different crystal forms, should help to clarify their role in cell-cycle control. RESULTS. We report the X-ray crystal structure of p13(suc1) to 1.95 A resolution in space group C2221. It is present in the crystals as a strand-exchanged dimer. The overall monomeric fold is preserved in each lobe of the dimer but a single beta-strand (Ile94-Asp102) is exchanged between the central beta-sheets of each molecule. CONCLUSIONS. Strand exchange, which has been observed for p13(suc1) in two different space groups, and for CksHs2, is now confirmed to be an intrinsic feature of the CKS family. A switch between levels of assembly may serve to coordinate the function of the CKS proteins in cell-cycle control.

Crystal structure of the **yeast** cell-cycle control protein, p13suc1, in a strand-exchanged dimer.
Mar 15 **1996**,
BACKGROUND. p13(suc1) from fission **yeast** is a member of the CDC28 kinase specific (CKS) class of cell-cycle control proteins, that includes **CKS1** from budding **yeast** and the **human** homologues CksHs1 and CksHs2. p13(suc1) participates in the regulation of p34(cdc2), a **cyclin-dependent kinase** controlling the G1-S and the G2-M transitions of the cell cycle. The CKS...

11/3,K,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12930737 PMID: 8601310
Crystal structure and mutational analysis of the **human** CDK2 kinase complex with cell cycle-regulatory protein CksHs1.
Bourne Y; Watson M H; Hickey M J; Holmes W; Rocque W; Reed S I; Tainer J A
Department of Molecular Biology, Scripps Research Institute, La Jolla, California, 92037, USA.
Cell (UNITED STATES) Mar 22 **1996**, 84 (6) p863-74, ISSN 0092-8674 Journal Code: 0413066
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The 2.6 Angstrom crystal structure for **human cyclin-dependent kinase** 2(CDK2) in complex with CksHs1, a **human** homolog of essential **yeast** cell cycle-regulatory proteins suc1 and **Cks1** , reveals that CksHs1 binds via all four beta strands to the kinase C-terminal lobe. This interface is biologically critical, based upon mutational analysis, but far from the CDK2 N-terminal lobe, cyclin, and regulatory phosphorylation sites. CDK2 binds the Cks single domain conformation and interacts with conserved hydrophobic residues plus His-60 and Glu-63 in their closed beta-hinge motif conformation. The beta hinge opening to form the Cks beta-interchanged dimer sterically precludes CDK2 binding, providing a possible mechanism regulating CDK2-Cks interactions. One face of the complex exposes the sequence-conserved phosphate-binding region on Cks and the ATP-binding site on CDK2, suggesting that CKs may target CDK2 to other phosphoproteins during the cell cycle.

Crystal structure and mutational analysis of the **human** CDK2 kinase complex with cell cycle-regulatory protein CksHs1.
Mar 22 **1996**,
The 2.6 Angstrom crystal structure for **human cyclin-dependent kinase** 2(CDK2) in complex with CksHs1, a **human** homolog of essential **yeast** cell cycle-regulatory proteins suc1 and **Cks1** , reveals that CksHs1 binds via all four beta strands to the kinase C-terminal lobe...
Tags: **Human**;

11/3,K,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11218146 PMID: 11231585
The cell-cycle regulatory protein **Cks1** is required for SCF(Skp2)-mediated ubiquitinylation of p27.
Ganoth D; Bornstein G; Ko T K; Larsen B; Tyers M; Pagano M; Hershko A
Unit of Biochemistry, B. Rappaport Faculty of Medicine, Technion-Israel

Institute of Technology, Haifa, 31096, Israel.
Nature cell biology (England) Mar **2001**, 3 (3) p321-4, ISSN 1465-7392 Journal Code: 100890575
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The **cyclin-dependent kinase** (CDK) inhibitor p27 is degraded in late G1 phase by the ubiquitin pathway, allowing CDK activity to drive cells into S phase. Ubiquitinylation of p27 requires its phosphorylation at Thr 187 (refs 3, 4) and subsequent recognition by S-phase kinase associated protein 2 (Skp2; refs 5-8), a member of the F-box family of proteins that associates with Skp1, Cul-1 and ROC1/Rbx1 to form an SCF ubiquitin ligase complex. However, in vitro ligation of p27 to ubiquitin could not be reconstituted by known purified components of the SCFSkp2 complex. Here we show that the missing factor is CDK subunit 1 (**Cks1**), which belongs to the highly conserved Suc1/Cks family of proteins that bind to some CDKs and phosphorylated proteins and are essential for cell-cycle progression. **Human Cks1**, but not other members of the family, reconstitutes ubiquitin ligation of p27 in a completely purified system, binds to Skp2 and greatly increases binding of T187-phosphorylated p27 to Skp2. Our results represent the first evidence that an SCF complex requires an accessory protein for activity as well as for binding to its phosphorylated substrate.

The cell-cycle regulatory protein **Cks1** is required for SCF(Skp2)-mediated ubiquitinylation of p27.
Mar **2001**,
The **cyclin-dependent kinase** (CDK) inhibitor p27 is degraded in late G1 phase by the ubiquitin pathway, allowing CDK...

... of the SCFSkp2 complex. Here we show that the missing factor is CDK subunit 1 (**Cks1**), which belongs to the highly conserved Suc1/Cks family of proteins that bind to some CDKs and phosphorylated proteins and are essential for cell-cycle progression. **Human Cks1**, but not other members of the family, reconstitutes ubiquitin ligation of p27 in a completely...
Tags: **Human**;

11/3,K,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10863651 PMID: 10997903
Crystal structure and mutational analysis of the Saccharomyces cerevisiae cell cycle regulatory protein **Cks1**: implications for domain swapping, anion binding and protein interactions.
Bourne Y; Watson M H; Arvai A S; Bernstein S L; Reed S I; Tainer J A
Centre National de la Recherche Scientifique, Marseille, France. yves@afmb.cnrs-mrs.fr
Structure with Folding & design (ENGLAND) Aug 15 **2000**, 8 (8) p841-50, ISSN 0969-2126 Journal Code: 100889329
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: The Saccharomyces cerevisiae protein **Cks1** (**cyclin -dependent kinase** subunit 1) is essential for cell-cycle progression. The biological function of **Cks1** can be modulated by a switch between two distinct molecular assemblies: the single domain fold, which results from the closing of a beta-hinge motif, and the intersubunit beta-strand interchanged dimer, which arises from the opening of the beta-hinge motif. The crystal structure of a **cyclin-dependent**

kinase (Cdk) in complex with the **human** Cks homolog CksHs1 single-domain fold revealed the importance of conserved hydrophobic residues and charged residues within the beta-hinge motif. RESULTS: The 3.0 A resolution **Cks1** structure reveals the strict structural conservation of the Cks alpha/beta-core fold and the beta-hinge motif. The beta hinge identified in the **Cks1** structure includes a novel pivot and exposes a cluster of conserved tyrosine residues that are involved in Cdk binding but are sequestered in the beta-interchanged Cks homolog suc1 dimer structure. This **Cks1** structure confirms the conservation of the Cks anion-binding site, which interacts with sidechain residues from the C-terminal alpha helix of another subunit in the crystal. CONCLUSIONS: The **Cks1** structure exemplifies the conservation of the beta-interchanged dimer and the anion-binding site in evolutionarily distant **yeast** and **human** Cks homologs. Mutational analyses including in vivo rescue of **CKS1** disruption support the dual functional roles of the beta-hinge residue Glu94, which participates in Cdk binding, and of the anion-binding pocket that is located 22 A away and on an opposite face to Glu94. The **Cks1** structure suggests a biological role for the beta-interchanged dimer and the anion-binding site in targeting Cdks to specific phosphoproteins during cell-cycle progression.

Crystal structure and mutational analysis of the Saccharomyces cerevisiae cell cycle regulatory protein **Cks1**: implications for domain swapping, anion binding and protein interactions.
Aug 15 **2000**,
BACKGROUND: The Saccharomyces cerevisiae protein **Cks1** (**cyclin -dependent kinase** subunit 1) is essential for cell-cycle progression. The biological function of **Cks1** can be modulated by a switch between two distinct molecular assemblies: the single domain fold...

... which arises from the opening of the beta-hinge motif. The crystal structure of a **cyclin-dependent kinase** (Cdk) in complex with the **human** Cks homolog CksHs1 single-domain fold revealed the importance of conserved hydrophobic residues and charged residues within the beta-hinge motif. RESULTS: The 3.0 A resolution **Cks1** structure reveals the strict structural conservation of the Cks alpha/beta-core fold and the beta-hinge motif. The beta hinge identified in the **Cks1** structure includes a novel pivot and exposes a cluster of conserved tyrosine residues that are...

... Cdk binding but are sequestered in the beta-interchanged Cks homolog suc1 dimer structure. This **Cks1** structure confirms the conservation of the Cks anion-binding site, which interacts with sidechain residues from the C-terminal alpha helix of another subunit in the crystal. CONCLUSIONS: The **Cks1** structure exemplifies the conservation of the beta-interchanged dimer and the anion-binding site in evolutionarily distant **yeast** and **human** Cks homologs. Mutational analyses including in vivo rescue of **CKS1** disruption support the dual functional roles of the beta-hinge residue Glu94, which participates in...

...pocket that is located 22 A away and on an opposite face to Glu94. The **Cks1** structure suggests a biological role for the beta-interchanged dimer and the anion-binding site...
Tags: **Human**;
Chemical Name: Anions; **CKS1** protein, S cerevisiae; Cell Cycle Proteins; Fungal Proteins; Saccharomyces cerevisiae Proteins

11/3,K,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10791013 PMID: 10913169
Cks1 is required for G(1) cyclin-**cyclin-dependent**

kinase activity in budding **yeast**.
Reynard G J; Reynolds W; Verma R; Deshaies R J
Division of Biology, California Institute of Technology, Pasadena, California 91125, USA.
Molecular and cellular biology (UNITED STATES) Aug **2000**, 20 (16) p5858-64, ISSN 0270-7306 Journal Code: 8109087
Contract/Grant No.: RO1 GM52466; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
p13(suc1) (Cks) proteins have been implicated in the regulation of **cyclin-dependent kinase** (CDK) activity. However, the mechanism by which Cks influences the function of cyclin-CDK complexes has remained elusive. We show here that **Cks1** is required for the protein kinase activity of budding **yeast** G(1) cyclin-CDK complexes. Cln2 and Cdc28 subunits coexpressed in baculovirus-infected insect cells fail to exhibit protein kinase activity towards multiple substrates in the absence of **Cks1**. **Cks1** can both stabilize Cln2-Cdc28 complexes and activate intact complexes in vitro, suggesting that it plays multiple roles in the biogenesis of active G(1) cyclin-CDK complexes. In contrast, Cdc28 forms stable, active complexes with the B-type cyclins Clb4 and Clb5 regardless of whether **Cks1** is present. The levels of Cln2-Cdc28 and Cln3-Cdc28 protein kinase activity are severely reduced in **cks1**-38 cell extracts. Moreover, phosphorylation of G(1) cyclins, which depends on Cdc28 activity, is reduced in **cks1**-38 cells. The role of **Cks1** in promoting G(1) cyclin-CDK protein kinase activity both in vitro and in vivo provides a simple molecular rationale for the essential role of **CKS1** in progression through G(1) phase in budding **yeast**.

Cks1 is required for G(1) cyclin-**cyclin-dependent kinase** activity in budding **yeast**.
Aug **2000**,
p13(suc1) (Cks) proteins have been implicated in the regulation of **cyclin-dependent kinase** (CDK) activity. However, the mechanism by which Cks influences the function of cyclin-CDK complexes has remained elusive. We show here that **Cks1** is required for the protein kinase activity of budding **yeast** G(1) cyclin-CDK complexes. Cln2 and Cdc28 subunits coexpressed in baculovirus-infected insect cells fail to exhibit protein kinase activity towards multiple substrates in the absence of **Cks1**. **Cks1** can both stabilize Cln2-Cdc28 complexes and activate intact complexes in vitro, suggesting that it...

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Chemical Name: **CKS1** protein, S cerevisiae; Cell Cycle Proteins; Cyclins; Fungal Proteins; Saccharomyces cerevisiae Proteins; Cyclin-Dependent Kinases

11/3,K,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10616248 PMID: 10722661
Isolation of Trypanosoma brucei CYC2 and CYC3 cyclin genes by rescue of a **yeast** G(1) cyclin mutant. Functional characterization of CYC2.
Van Hellemond J J; Neuville P; Schwarz R T; Matthews K R; Mottram J C

Wellcome Centre for Molecular Parasitology, University of Glasgow, Anderson College, Glasgow G11 6NU, Scotland, United Kingdom.
Journal of biological chemistry (UNITED STATES) Mar 24 **2000**, 275 (12) p8315-23, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Two Trypanosoma brucei cyclin genes, CYC2 and CYC3, have been isolated by rescue of the Saccharomyces cerevisiae mutant DL1, which is deficient in CLN G(1) cyclin function. CYC2 encodes a 24-kDa protein that has sequence identity to the Neurospora crassa PREG1 and the S. cerevisiae PHO80 cyclin. CYC3 has the most sequence identity to mitotic B-type cyclins from a variety of organisms. Both CYC2 and CYC3 are single-copy genes and expressed in all life cycle stages of the parasite. To determine if CYC2 is found in a complex with previously identified trypanosome cdc2-related kinases (CRKs), the CYC2 gene was fused to the TY epitope tag, integrated into the trypanosome genome, and expressed under inducible control. CYC2ty was found to associate with an active trypanosome CRK complex since CYC2ty bound to leishmanial p12(**cks1**), and histone H1 kinase activity was detected in CYC2ty immune-precipitated fractions. Gene knockout experiments provide evidence that CYC2 is an essential gene, and co-immune precipitations together with a two-hybrid interaction assay demonstrated that CYC2 interacts with CRK3. The CRK3 x CYC2ty complex, the first **cyclin-dependent kinase** complex identified in trypanosomes, was localized by immune fluorescence to the cytoplasm throughout the cell cycle.

Isolation of Trypanosoma brucei CYC2 and CYC3 cyclin genes by rescue of a **yeast** G(1) cyclin mutant. Functional characterization of CYC2.
Mar 24 **2000**,

...found to associate with an active trypanosome CRK complex since CYC2ty bound to leishmanial p12(**cks1**), and histone H1 kinase activity was detected in CYC2ty immune-precipitated fractions. Gene knockout experiments ...

...interaction assay demonstrated that CYC2 interacts with CRK3. The CRK3 x CYC2ty complex, the first **cyclin-dependent kinase** complex identified in trypanosomes, was localized by immune fluorescence to the cytoplasm throughout the cell...

...Chemical Name: Proteins; Schizosaccharomyces pombe Proteins; Suc1 protein, S pombe; cyc2 protein, Paramecium tetraurelia; cyclin G1; p12 **cks1** protein, Leishmania mexicana; Cyclin-Dependent Kinases

11/3,K,AB/10 (Item 1 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

0013172628 BIOSIS NO.: 200100344467
Protein destruction: Adapting roles for Cks proteins
AUTHOR: Harper J Wade (Reprint)
AUTHOR ADDRESS: Department of Biochemistry and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX, 77030, USA**USA
JOURNAL: Current Biology 11 (11): pR431-R435 5 June, 2001 **2001**
MEDIUM: print
ISSN: 0960-9822
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: **Cks1**, a subunit of cyclin-dependent kinases, has now been identified as an essential cofactor in the ubiquitination of the Cdk inhibitor p27 by the SCFSkp2 ubiquitin ligase. This activity, which can

be independent of Cdk binding, links Cks to positive growth control pathways regulating the G1/S transition and to cancer.

2001
ABSTRACT: **Cks1**, a subunit of cyclin-dependent kinases, has now been identified as an essential cofactor in...
...REGISTRY NUMBERS: **cyclin-dependent kinase**
DESCRIPTORS:
ORGANISMS: fission **yeast** (Ascomycetes...

...**yeast** (Fungi
CHEMICALS & BIOCHEMICALS: **Cks1**; ...

...**cyclin-dependent kinase**;

11/3,K,AB/11 (Item 2 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

0013062952 BIOSIS NO.: 200100234791
A CDK-independent function of mammalian **Cks1**: Targeting of SCFSkp2 to the CDK inhibitor p27Kip1
AUTHOR: Spruck Charles; Strohmaier Heimo; Watson Mark; Smith Adrian P L; Ryan Aimee; Krek Wilhelm; Reed Steven I (Reprint)
AUTHOR ADDRESS: Scripps Research Institute, La Jolla, CA, 92037, USA**USA
JOURNAL: Molecular Cell 7 (3): p639-650 March, 2001 **2001**
MEDIUM: print
ISSN: 1097-2765
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The Cks/Suc1 proteins associate with CDK/cyclin complexes, but their precise function(s) is not well defined. Here we demonstrate that **Cks1** directs the ubiquitin-mediated proteolysis of the CDK-bound substrate p27Kip1 by the protein ubiquitin ligase (E3) SCFSkp2. **Cks1** associates with the F box protein Skp2 and is essential for recognition of the p27Kip1 substrate for ubiquitination in vivo and in vitro. Using purified recombinant proteins, we reconstituted p27Kip1 ubiquitination activity and show that it is dependent on **Cks1**. **CKS1**-/- mice are abnormally small, and cells derived from them proliferate poorly, particularly under limiting mitogen conditions, possibly due to elevated levels of p27Kip1.

A CDK-independent function of mammalian **Cks1**: Targeting of SCFSkp2 to the CDK inhibitor p27Kip1
2001

...ABSTRACT: cyclin complexes, but their precise function(s) is not well defined. Here we demonstrate that **Cks1** directs the ubiquitin-mediated proteolysis of the CDK-bound substrate p27Kip1 by the protein ubiquitin ligase (E3) SCFSkp2. **Cks1** associates with the F box protein Skp2 and is essential for recognition of the p27Kip1...

...purified recombinant proteins, we reconstituted p27Kip1 ubiquitination activity and show that it is dependent on **Cks1**. **CKS1**-/- mice are abnormally small, and cells derived from them proliferate poorly, particularly under limiting mitogen...
DESCRIPTORS:
ORGANISMS: **mouse** (Muridae...

...**CKS1** double-negative
CHEMICALS & BIOCHEMICALS: mammalian **Cks1**--...

...CDK inhibitor, **cyclin-dependent kinase** inhibitor

11/3,K,AB/12 (Item 3 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

0011804356 BIOSIS NO.: 199900064016
Regulation of Cdc28 **cyclin-dependent** protein **kinase** activity during the cell cycle of the **yeast** Saccharomyces cerevisiae
AUTHOR: Mendenhall Michael D (Reprint); Hodge Amy E
AUTHOR ADDRESS: L.P. Markey Cancer Center, University Kentucky, D. E. Combs Building, Room 212, 800 Rose St., Lexington, KY 40536-0096, USA**USA
JOURNAL: Microbiology and Molecular Biology Reviews 62 (4): p1191-1243 Dec., 1998 **1998**
MEDIUM: print
ISSN: 1092-2172
DOCUMENT TYPE: Article; Literature Review
RECORD TYPE: Citation
LANGUAGE: English

Regulation of Cdc28 **cyclin-dependent** protein **kinase** activity during the cell cycle of the **yeast** Saccharomyces cerevisiae
1998
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ...Cdc28 **cyclin-dependent** protein **kinase**--...

...**Cks1**--

11/3,K,AB/13 (Item 4 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

0011462104 BIOSIS NO.: 199800256351
The crk3 gene of Leishmania mexicana encodes a stage-regulated cdc2-related histone H1 kinase that associates with p12cks1
AUTHOR: Grant Karen M; Hassan Paul; Anderson J Simon; Mottram Jeremy C (Reprint)
AUTHOR ADDRESS: Wellcome Unit Mol. Parasitol., Univ. Glasgow, Anderson Coll., 56 Dumbarton Rd., Glasgow G11 6NU, UK**UK
JOURNAL: Journal of Biological Chemistry 273 (17): p10153-10159 April 24, 1998 **1998**
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A cdc2-related protein kinase gene, crk3, has been isolated from the Parasitic Protozoan Leishmania mexicana. Data presented here suggests that crk3 is a good candidate to be the leishmanial cdc2 homologue but that the parasite protein has some characteristics which distinguish it from mammalian cdc2. crk3 is predicted to encode a 35.6-kDa protein with 54% sequence identity With the **human cyclin-dependent kinase** cdc2 and 78% identity with the Trypanosoma brucei CRK3. The trypanosomatid CRK3 proteins have an unusual, poorly conserved 19-amino acid N-terminal extension not present in **human** cdc2. crk3 is single

copy, and there is 5-fold higher mRNA in the replicative promastigote life-cycle stage than in the non-dividing metacyclic form or mammalian amastigote form. A leishmanial suc-binding cdc2-related kinase (SBCRK) histone H1 kinase, has previously been described which binds the **yeast** protein, p13suc1, and that has stage-regulated activity (Mottram J. C., Kinnaird, J., Shiels, B. R., Tait, A., and Barry, J. D. (1993) J. Biol. Chem. 268, 21044-21051). CRK3 from cell extracts of the three life-cycle stages was found to bind p13suc1 and the leishmanial homologue p12cks1. CRK3 fused with six histidines at the C terminus was expressed in L. mexicana and shown to have SBCRK histone H1 kinase activity. Depletion of histidine-tagged CRK3 from L. mexicana cell extracts, by Ni-nitrilotriacetic acid agarose selection, reduced histone H1 kinase activity binding to p13suc1. These data imply that crk3 encodes the kinase subunit of SBCRK. SBCRK and histidine-tagged CRK3 activities were inhibited by the purine analogue olomoucine with an IC50 of 28 and 42 muM, respectively, 5-6-fold higher than **human** p34cdc2/cyclinB.

1998

...ABSTRACT: is predicted to encode a 35.6-kDa protein with 54% sequence identity With the **human cyclin-dependent kinase** cdc2 and 78% identity with the Trypanosoma brucei CRK3. The trypanosomatid CRK3 proteins have an unusual, poorly conserved 19-amino acid N-terminal extension not present in **human** cdc2. crk3 is single copy, and there is 5-fold higher mRNA in the replicative...

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...olomoucine with an IC50 of 28 and 42 muM, respectively, 5-6-fold higher than **human** p34cdc2/cyclinB.
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ...**human** cdc2...

...**human** p34-cdc2/cyclin B...

...p12-**cks1**;

11/3,K,AB/14 (Item 5 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

0011078445 BIOSIS NO.: 199799712505
The Arabidopsis CKs1At protein binds the cyclin-dependent kinases Cdc2aAT and Cdc2bAt
AUTHOR: De Veylder Lieven; Segers Gerda; Glab Nathalie; Casteels Peter; Van Montagu Marc (Reprint); Inze Dirk
AUTHOR ADDRESS: Lab. Genetica, Dep. Genetics, Flanders Interuniversity Inst. Biotechnol., Univ. Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium**Belgium
JOURNAL: FEBS Letters 412 (3): p446-452 1997 **1997**
ISSN: 0014-5793
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: In Arabidopsis, two cyclin-dependent kinases (CDK), Cdc2aAt and Cdc2bAt, have been described. Here, we have used the **yeast** two-hybrid system to identify Arabidopsis proteins interacting with Cdc2aAt. Three different clones were isolated, one of which encodes a Suc1/**Cks1** homologue. The functionality of the Arabidopsis Suc1/

Cks1 homologue, designed Cks1At, was demonstrated by its ability to rescue the temperature-sensitive cdc2-L7 strain of fission **yeast** at low and intermediate expression levels. In contrast, high cks1At expression levels inhibited cell division in both mutant and wild-type **yeast** strains. Cks1At binds both Cdc2aAt and Cdc2bAt in vivo and in vitro. Furthermore, we demonstrate that the fission **yeast** Suc1 binds Cdc2aAt but only weakly Cdc2bAt, whereas the **human** CksHs1 associated exclusively with Cdc2aAt.

1997

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DESCRIPTORS:
MISCELLANEOUS TERMS: ...**CYCLIN-DEPENDENT KINASE** CDC2AAT...

...**CYCLIN-DEPENDENT KINASE** CDC2BAT...

...**YEAST** TWO-HYBRID SCREEN

11/3,K,AB/15 (Item 6 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

0010033856 BIOSIS NO.: 199598501689
P19-Skp1 and p45-Skp2 are essential elements of the cyclin A-CDK2 S phase kinase
AUTHOR: Zhang Hui; Kobayashi Ryuji; Galaktionov Konstantin; Beach David
AUTHOR ADDRESS: Howard Hughes Med. Inst., Cold Spring Harbor Lab., Cold Spring Harbor, NY 11724, USA**USA
JOURNAL: Cell 82 (6): p915-925 1995 **1995**
ISSN: 0092-8674
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: In normal **human** fibroblasts, cyclin A-CDK2 exists in a quaternary complex that contains p21 and PCNA. In many transformed cells, p22 disappears, and a substantial fraction of cyclin A-CDK2 complexes with p9-**CKS1/CKS2**, p19, and p45. To investigate the significance of these rearrangements, we have isolated cDNAs encoding p19 and p45. In vitro reconstitution demonstrated that binding of p19 to cyclin A-CDK2 requires p45. Addition of these proteins to the kinase had no substantial effect on the kinase activity in vitro. Interference with p45 function in vivo by microinjection of antibodies or antisense oligonucleotides prevented entry into S phase in both normal and transformed cells. Cyclin A-CDK2 has previously been identified as a kinase whose activity is essential for S phase. Our results identify p45 as an essential element of this activity. The abundance of p45 is greatly increased in many transformed cells. This could result in changes in cell

cycle control that contribute to the process of cellular transformation.

1995

ABSTRACT: In normal **human** fibroblasts, cyclin A-CDK2 exists in a quaternary complex that contains p21 and PCNA. In...

...transformed cells, p22 disappears, and a substantial fraction of cyclin A-CDK2 complexes with p9-**CKS1**/**CKS2**, p19, and p45. To investigate the significance of these rearrangements, we have isolated cDNAs encoding...
DESCRIPTORS:
...MAJOR CONCEPTS: **Human** Medicine, Medical Sciences
MISCELLANEOUS TERMS: ...**CYCLIN-DEPENDENT KINASE** 2...

...**HUMAN** FIBROBLASTS

11/3,K,AB/16 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

09217172 Genuine Article#: 380KY Number of References: 23
Title: Trypanosoma brucei CYC1 does not have characteristics of a mitotic cyclin
Author(s): Hammarton TC; Ford JR; Mottram JC (REPRINT)
Corporate Source: UNIV GLASGOW,ANDERSON COLL, WELLCOME CTR MOL PARASITOL/GLASGOW G11 6NU/LANARK/SCOTLAND/ (REPRINT); UNIV GLASGOW,ANDERSON COLL, WELLCOME CTR MOL PARASITOL/GLASGOW G11 6NU/LANARK/SCOTLAND/
Journal: MOLECULAR AND BIOCHEMICAL PARASITOLOGY, **2000**, V111, N1 (NOV), P229-234
ISSN: 0166-6851 Publication date: 20001100
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS
Language: English Document Type: ARTICLE

, **2000**
...Identifiers--CDC2-RELATED PROTEIN-KINASES; HISTONE H1 KINASE; LEISHMANIA-MEXICANA; **YEAST**; HOMOLOG; COMPLEMENTATION; EXPRESSION; P12(**CKS1**); MECHANISM

11/3,K,AB/17 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

04959293 Genuine Article#: UV483 Number of References: 47
Title: LEISHMANIA-MEXICANA P12(**CKS1**), A HOMOLOG OF FISSION **YEAST** P13(SUC1), ASSOCIATES WITH A STAGE-REGULATED HISTONE H1 KINASE (Abstract Available)
Author(s): MOTTRAM JC; GRANT KM
Corporate Source: UNIV GLASGOW,ANDERSON COLL,WELLCOME UNIT MOLEC PARASITOL,56 DUMBARTON RD/GLASGOW G11 6NU/LANARK/SCOTLAND/
Journal: BIOCHEMICAL JOURNAL, **1996**, V316, JUN (JUN 15), P833-839
ISSN: 0264-6021
Language: ENGLISH Document Type: ARTICLE
Abstract: We have isolated a Leishmania mexicana homologue of the fission **yeast** suc1 gene using PCR with oligonucleotides designed to conserved regions of cdc2 kinase subunits (cks). Tile product of **cks1** is a 12 kDa polypeptide, which has 70% identity with **human** p9(**cks1**) and 44% identity with fission **yeast** p13(suc1). p12(**cks1**) was detected in the three life-cycle stages of L. mexicana by immunoblotting. Recombinant p12(**cks1**)

(p12(cks1his)) bound to agarose beads was used as a matrix to affinity-select histone III kinase complexes from Leishmania, **yeast** and bovine extracts. Immunoblotting showed that **yeast** and bovine cdc2 kinase bound to p12(cks1his), thus demonstrating functional homology between L. mexicana p12(**cks1**) and **yeast** p13(suc1). Histone H1 kinase activity was found at a high level in the proliferative promastigote and amastigote forms of L. mexicana, but at a low level in the non-dividing metacyclic form. These activities are likely to be the same as the leishmanial p13(suc1) binding kinase (SBCRK) described previously [Mottram, Kinnaird, Shiels, Tait and Barry (1993) J. Biol. Chem. 268, 21044-21051]. A distinct cdc2-related kinase, L. mexicana CRK1, was also found to associate with p12(cks1his) but affinity-depletion experiments showed that CRK1 was not responsible for the histone I-Il kinase activity associating with p12(cks1his) in promastigote cell extracts. The finding that p12(**cks1**) associates with at least two cdc2-related kinases, SBCRK and CRK1, is consistent with the presence of a large gene family of cdc2-related kinases in trypanosomatids, a situation thought to be more similar to higher eukaryotes than **yeast**.

Title: LEISHMANIA-MEXICANA P12(**CKS1**), A HOMOLOG OF FISSION **YEAST** P13(SUC1), ASSOCIATES WITH A STAGE-REGULATED HISTONE H1 KINASE
, **1996**
Abstract: We have isolated a Leishmania mexicana homologue of the fission **yeast** suc1 gene using PCR with oligonucleotides designed to conserved regions of cdc2 kinase subunits (cks). Tile product of **cks1** is a 12 kDa polypeptide, which has 70% identity with **human** p9(**cks1**) and 44% identity with fission **yeast** p13(suc1). p12(**cks1**) was detected in the three life-cycle stages of L. mexicana by immunoblotting. Recombinant p12(**cks1**) (p12(cks1his)) bound to agarose beads was used as a matrix to affinity-select histone III kinase complexes from Leishmania, **yeast** and bovine extracts. Immunoblotting showed that **yeast** and bovine cdc2 kinase bound to p12(cks1his), thus demonstrating functional homology between L. mexicana p12(**cks1**) and **yeast** p13(suc1). Histone H1 kinase activity was found at a high level in the proliferative...

...Il kinase activity associating with p12(cks1his) in promastigote cell extracts. The finding that p12(**cks1**) associates with at least two cdc2-related kinases, SBCRK and CRK1, is consistent with the...

...related kinases in trypanosomatids, a situation thought to be more similar to higher eukaryotes than **yeast**.
Research Fronts: 94-1665 001 (**CYCLIN-DEPENDENT KINASE**-4 INHIBITOR GENE; GERMLINE P16 MUTATIONS IN FAMILIAL MELANOMA; HOMOZYGOUS DELETIONS)
94-4806 001 (GENE...

11/3,K,AB/18 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

04375351 Genuine Article#: RZ082 Number of References: 44
Title: TRANSFORMING GROWTH-FACTOR-BETA DOWN-REGULATION OF CKSHS1 TRANSCRIPTS IN GROWTH-INHIBITED EPITHELIAL-CELLS (Abstract Available)
Author(s): SIMON KE; CHA HH; FIRESTONE GL
Corporate Source: UNIV CALIF BERKELEY,DEPT MOLEC & CELL BIOL,BOX 591 LSA/BERKELEY//CA/94720; UNIV CALIF BERKELEY,DEPT MOLEC & CELL BIOL/BERKELEY//CA/94720; UNIV CALIF BERKELEY,CANC RES LAB/BERKELEY//CA/94720
Journal: CELL GROWTH & DIFFERENTIATION, **1995**, V6, N10 (OCT), P

1261-1269
ISSN: 1044-9523
Language: ENGLISH Document Type: ARTICLE
Abstract: CKShs1 is a mammalian homologue of the **yeast** suc1 and **CKS1** genes, for which the null mutation leads to arrest in both the G(1) and G(1) phases of the cell cycle in Saccharomyces cerevisiae. Northern blot analysis revealed that transcript levels of CKShs1 are strongly down-regulated in mink lung cells and moderately down-regulated in BALB keratinocytes within 10 h of exposure to transforming growth factor beta (TCF-beta), whereas growth arrest of both cell lines requires at least 15 h of TGF-beta treatment. As a genetic test for the potential role of CKShs1 in TGF-beta growth regulation, we analyzed a stably transfected derivative of mink lung cells that constitutively overexpresses a truncated form of the type 2 TGF-beta receptor and is resistant to TGF-beta growth inhibition; CKShs1 transcripts are not downregulated by TGF-beta in this mutant cell line. TGF-beta down-regulation of CKShs1 transcripts is specific, since mRNA levels of mammalian G1 cyclins D1, D2, and D3 do not change in response to TGF-beta in either cell line. Cyclin D1 and cyclin D2 transcripts are strongly induced by epidermal growth factor, and beta 2-microglobulin transcripts are strongly induced by TGF-beta in BALB keratinocytes released from quiescence by addition of epidermal growth factor. Our results suggest a role for CKShs1 gene products in TGF-beta growth arrest of epithelial cells.

, **1995**
Abstract: CKShs1 is a mammalian homologue of the **yeast** suc1 and **CKS1** genes, for which the null mutation leads to arrest in both the G(1) and...
...Identifiers--**CYCLIN-DEPENDENT** KINASES; CDC2 PROTEIN-**KINASE**; TGF-BETA; RETINOBLASTOMA PROTEIN; SACCHAROMYCES-CEREVISIAE; SCHIZOSACCHAROMYCES-POMBE; SUBUNIT; GENE; PHOSPHORYLATION; EXPRESSION

11/3,K,AB/19 (Item 1 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 3232692 IFI Acc No: 9939071
IFI Publication Control No: 9939071
Document Type: C
CYCLIN/CDK ASSOCIATED PROTEINS, AND USES RELATED THERETO; POLYPEPTIDE WHICH IS CAN BIND TO **CYCLIN-DEPENDENT KINASE**; FOR DIAGNOSIS AND THERAPY OF CELL PROLIFERATIVE DEFECTS IN ANIMALS; ANTIPROLIFERATIVE AGENTS
Inventors: Beach David (US); Zhang Hui (US)
Assignee: Cold Spring Harbor Laboratory
Assignee Code: 00797
Publication (No,Kind,Date), Applic (No,Date):
US 5981702 A **19991109** US 95531439 19950921
Calculated Expiration: 20150921
  Document Type: CERTIFICATE OF CORRECTION
Certificate of Correction Date: 20000523
Priority Applic(No,Date): US 95531439 19950921

Abstract: The present invention relates to the discovery in mammalian cells, particularly **human** cells, of novel S-phase kinase associated proteins, p19 and p45, referred to herein as "Skp". As described herein, these proteins are components of the tumor cell-specific cyclin A/CDK2 complex and function to facilitate DNA replication. Interference with p45 function in vivo prevented entry into S-phase in both normal and transformed cells. Binding data indicated that p45 and p19 associate with each other in a binary complex. Moreover, p45 is required for p19 binding to cyclin A/CDK2.

...POLYPEPTIDE WHICH IS CAN BIND TO **CYCLIN-DEPENDENT KINASE**; FOR DIAGNOSIS AND THERAPY OF CELL PROLIFERATIVE DEFECTS IN ANIMALS; ANTIPROLIFERATIVE AGENTS
Publication (No,Kind,Date), Applic (No,Date):
...**19991109**

Abstract: The present invention relates to the discovery in mammalian cells, particularly **human** cells, of novel S-phase kinase associated proteins, p19 and p45, referred to herein as...

Exemplary Claim: ...an independent binary complex or in association with at least one of a cyclin, a **cyclin-dependent kinase** (CDK) and a p9CKS1/**CKS2** polypeptide.
Non-exemplary Claims: ...an independent binary complex or in association with at least one of a cyclin, a **cyclin-dependent kinase** (CDK) and a p9CKS1/**CKS2** polypeptide...

...9. The p19 polypeptide of claim 2, wherein the mammalian p19 polypeptide is a **human** p19 polypeptide...
?

3/3,K,AB/8 (Item 1 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

0013330409 BIOSIS NO.: 200100502248
Isolation and characterization of **survivin-binding** proteins
AUTHOR: Wang Qiang (Reprint)
AUTHOR ADDRESS: University of Pennsylvania, Philadelphia, PA, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 42 p593 March, 2001 **2001**
MEDIUM: print
CONFERENCE/MEETING: 92nd Annual Meeting of the American Association for Cancer Research New Orleans, LA, USA March 24-28, 2001; 20010324
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

Isolation and characterization of **survivin-binding** proteins
2001
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: **survivin binding** proteins...

942716 PMID: 11084331

Survivin and the inner centromere protein INCENP show similar cell-cycle localization and gene knockout phenotype.

Uren A G; Wong L; Pakusch M; Fowler K J; Burrows F J; Vaux D L; Choo K H

The Walter and Eliza Hall Institute of Medical Research, Post Office Royal Melbourne Hospital, 3050,., Victoria, Australia.

Current biology - CB (ENGLAND) Nov 2 **2000**, 10 (21) p1319-28, ISSN 0960-9822 Journal Code: 9107782

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

BACKGROUND: Survivin is a mammalian protein that carries a motif typical of the inhibitor of apoptosis (IAP)proteins, first identified in baculoviruses. Although baculoviral IAP proteins regulate cell death, the yeast Survivin homolog Bir1 is involved in cell division. To determine the function of Survivin in mammals, we analyzed the pattern of localization of Survivin protein during the cell cycle, and deleted its gene by homologous recombination in mice. RESULTS: In human cells, Survivin appeared first on centromeres bound to a novel para-polar axis during prophase/metaphase, relocated to the spindle midzone during anaphase/telophase, and disappeared at the end of telophase. In the mouse, Survivin was required for mitosis during development. Null embryos showed disrupted microtubule formation, became polyploid, and failed to survive beyond 4.5days post coitum. This phenotype, and the cell-cycle localization of Survivin, resembled closely those of INCENP. Because the yeast homolog of INCENP, Sli15, regulates the Aurora kinase homolog Ipl1p, and the yeast **Survivin** homolog Bir1 **binds** to Ndc10p, a substrate of Ipl1p, yeast Survivin, INCENP and Aurora homologs function in concert during cell division. CONCLUSIONS: In vertebrates, Survivin and INCENP have related roles in mitosis, coordinating events such as microtubule organization, cleavage-furrow formation and cytokinesis. Like their yeast homologs Bir1 and Sli15, they may also act together with the Aurora kinase.

11419888 PMID: 11516652

INCENP is required for proper targeting of Survivin to the centromeres and the anaphase spindle during mitosis.

Wheatley S P; Carvalho A; Vagnarelli P; Earnshaw W C

Chromosome Structure Group, Institute of Cell and Molecular Biology, Swann Building, University of Edinburgh, King's Buildings, Mayfield Road, EH9 3JR, Edinburgh, United Kingdom.

Current biology - CB (England) Jun 5 **2001**, 11 (11) p886-90, ISSN 0960-9822 Journal Code: 9107782

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Three lines of investigation have suggested that interactions between Survivin and the chromosomal passenger proteins INCENP and Aurora-B kinase may be important for mitotic progression. First, interference with the function of Survivin/BIR1, INCENP, or Aurora-B kinase leads to similar defects in mitosis and cytokinesis [1-7] (see [8] for review). Second, INCENP and Aurora-B exist in a complex in Xenopus eggs [9] and in mammalian cultured cells [7]. Third, interference with Survivin or INCENP function causes Aurora-B kinase to be mislocalized in mitosis in both C. elegans and vertebrates [5, 7, 9]. Here, we provide evidence that Survivin, Aurora-B, and INCENP interact physically and functionally. Direct visualization of Survivin-GFP in mitotic cells reveals that it localizes identically to INCENP and Aurora-B. **Survivin binds** directly to both Aurora-B and INCENP in yeast two-hybrid and in vitro pull-down assays. The in vitro interaction between Survivin and Aurora-B is extraordinarily stable in that it resists 3 M NaCl. Finally, Survivin and INCENP interact functionally in vivo; in cells in which INCENP localization is disrupted, Survivin adheres to the chromosomes and no longer concentrates at the centromeres or transfers to the anaphase spindle midzone. Our data provide the first biochemical evidence that Survivin can interact directly with members of the chromosomal passenger complex.

DIALOG(R)File 155:MEDLINE(R)

14149991 PMID: 9850056
IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs.
Tamm I; Wang Y; Sausville E; Scudiero D A; Vigna N; Oltersdorf T; Reed J C
The Burnham Institute, La Jolla, California 92037, USA.
Cancer research (UNITED STATES) Dec 1 **1998**, 58 (23) p5315-20,
ISSN 0008-5472 Journal Code: 2984705R
Contract/Grant No.: AG-15402; AG; NIA
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Survivin is a member of the inhibitor of apoptosis protein (IAP) family. We investigated the antiapoptotic mechanism of Survivin, as well as its expression in 60 human tumor cell lines used for the National Cancer Institute's anticancer drug screening program. In cotransfection experiments, cell death induced by Bax or Fas (CD 95) was partially inhibited (mean +/- SD, 65% +/- 8%) by Survivin, whereas XIAP, another IAP family member, almost completely blocked cell death (93% +/- 4%) under the same conditions. Survivin and XIAP also protected 293 cells from apoptosis induced by overexpression of procaspase-3 and -7 and inhibited the processing of these zymogens into active caspases. In vitro binding experiments indicated that, like other IAP-family proteins, **Survivin binds** specifically to the terminal effector cell death proteases, caspase-3 and -7, but not to the proximal initiator protease caspase-8. Using a cell-free system in which cytosolic extracts were derived from control- or Survivin-transfected cells and where caspases were activated either by addition of cytochrome c and dATP or by adding recombinant active caspase-8, Survivin was able to substantially reduce caspase activity, as measured by cleavage of a tetrapeptide substrate, AspGluValAsp-aminofluorocoumarin. Similar results were obtained in intact cells when Survivin was overexpressed by gene transfection and caspase activation was induced by the anticancer drug etoposide. Survivin was expressed in all 60 cancer cell lines analyzed, with highest levels in breast and lung cancers and lowest levels in renal cancers. These findings indicate that Survivin, which is commonly expressed in human tumor cell lines, can bind the effector cell death proteases caspase-3 and -7 in vitro and inhibits caspase activity and cell death in cells exposed to diverse apoptotic stimuli. Although quantitative differences may exist, these observations suggest commonality in the mechanisms used by IAP-family proteins to suppress apoptosis.
Dec 1 **1998**,
... zymogens into active caspases. In vitro binding experiments indicated that, like other IAP-family proteins, **Survivin binds** specifically to the terminal effector cell death proteases, caspase-3 and -7, but not to...

3/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11762240 PMID: 11942622
Role of chromosomal passenger complex in chromosome segregation and cytokinesis.
Terada Y
Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis 55455, USA. terad002@tc.umn.edu
Cell structure and function (Japan) Dec **2001**, 26 (6) p653-7,
ISSN 0386-7196 Journal Code: 7608465

Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Chromosomal passenger proteins associate with chromosomes early in

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? ds

Set     Items   Description
S1         85   SURVIVIN(5N)BIND?
S2         42   RD (unique items)
S3          9   S2 AND PY<=2001
S4      25728   CYCLIN(W)DEPENDENT(5N) KINASE
S5     105149   REGULAT?(5N)(DOMAIN?? OR REGION?? OR SUBUNIT?? OR AREA??)
S6        915   S4 AND S5
S7        208   CKS1 OR CKS2
S8        111   RD (unique items)
S9         45   S8 AND PY<2001
? s s6 and s7
             915  S6
             208  S7
     S10       4  S6 AND S7
? rd
>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
     S11       2  RD (unique items)
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11/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10863651 PMID: 10997903
Crystal structure and mutational analysis of the Saccharomyces cerevisiae cell cycle **regulatory** protein **Cks1**: implications for **domain** swapping, anion binding and protein interactions.
Bourne Y; Watson M H; Arvai A S; Bernstein S L; Reed S I; Tainer J A
Centre National de la Recherche Scientifique, Marseille, France. yves@afmb.cnrs-mrs.fr
Structure with Folding & design (ENGLAND) Aug 15 2000, 8 (8) p841-50 , ISSN 0969-2126 Journal Code: 100889329
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: The Saccharomyces cerevisiae protein **Cks1** (**cyclin -dependent kinase** subunit 1) is essential for cell-cycle progression. The biological function of **Cks1** can be modulated by a switch between two distinct molecular assemblies: the single domain fold, which results from the closing of a beta-hinge motif, and the intersubunit beta-strand interchanged dimer, which arises from the opening of the beta-hinge motif. The crystal structure of a **cyclin-dependent kinase** (Cdk) in complex with the human Cks homolog CksHs1 single-domain fold revealed the importance of conserved hydrophobic residues and charged residues within the beta-hinge motif. RESULTS: The 3.0 A resolution **Cks1** structure reveals the strict structural conservation of the Cks alpha/beta-core fold and the beta-hinge motif. The beta hinge identified in the **Cks1** structure includes a novel pivot and exposes a cluster of conserved tyrosine residues that are involved in Cdk binding but are sequestered in the beta-interchanged Cks homolog suc1 dimer structure. This **Cks1** structure confirms the conservation of the Cks anion-binding site, which interacts with sidechain residues from the C-terminal alpha helix of another subunit in the crystal. CONCLUSIONS: The **Cks1** structure exemplifies the conservation of the beta-interchanged dimer and the anion-binding site in evolutionarily distant yeast and human Cks homologs. Mutational analyses including in vivo rescue of **CKS1** disruption support the dual functional roles of the beta-hinge residue Glu94, which participates in Cdk binding, and of the anion-binding pocket that is located 22 A away and on an opposite face to Glu94. The **Cks1** structure suggests a biological role for the beta-interchanged dimer and the anion-binding site in targeting Cdks to specific phosphoproteins during cell-cycle progression.

Crystal structure and mutational analysis of the Saccharomyces cerevisiae cell cycle **regulatory** protein **Cks1**: implications for **domain** swapping, anion binding and protein interactions.
BACKGROUND: The Saccharomyces cerevisiae protein **Cks1** (**cyclin -dependent kinase** subunit 1) is essential for cell-cycle progression. The biological function of **Cks1** can be modulated by a switch between two distinct molecular assemblies: the single domain fold...

... which arises from the opening of the beta-hinge motif. The crystal structure of a **cyclin-dependent kinase** (Cdk) in complex with the human Cks homolog CksHs1 single-domain fold revealed the importance...

... residues and charged residues within the beta-hinge motif. RESULTS: The 3.0 A resolution **Cks1** structure reveals the strict structural conservation of the Cks alpha/beta-core fold and the beta-hinge motif. The beta hinge identified in the **Cks1** structure includes a novel pivot and exposes a cluster of conserved tyrosine residues that are...

... Cdk binding but are sequestered in the beta-interchanged Cks homolog suc1 dimer structure. This **Cks1** structure confirms the conservation of the Cks anion-binding site, which interacts with sidechain residues from the C-terminal alpha helix of another subunit in the crystal. CONCLUSIONS: The **Cks1** structure exemplifies the conservation of the beta-interchanged dimer and the anion-binding site in evolutionarily distant yeast and human Cks homologs. Mutational analyses including in vivo rescue of **CKS1** disruption support the dual functional roles of the beta-hinge residue Glu94, which participates in...

...pocket that is located 22 A away and on an opposite face to Glu94. The **Cks1** structure suggests a biological role for the beta-interchanged dimer and the anion-binding site...

Chemical Name: Anions; **CKS1** protein, S cerevisiae; Cell Cycle Proteins; Fungal Proteins; Saccharomyces cerevisiae Proteins

?

03499142 Genuine Article#: PJ285 Number of References: 18
Title: P35 IS A NEURAL-SPECIFIC **REGULATORY** SUBUNIT OF **CYCLIN-DEPENDENT KINASE**-5 (Abstract Available)
Author(s): TSAI LH; DELALLE I; CAVINESS VS; CHAE T; HARLOW E
Corporate Source: HARVARD UNIV,SCH MED,DEPT PATHOL,200 LONGWOOD AVE/BOSTON//MA/02115; HARVARD UNIV,MASSACHUSETTS GEN HOSP,SCH MED,DEPT NEUROL/BOSTON//MA/02114; MASSACHUSETTS GEN HOSP,CTR CANC/BOSTON//MA/02129
Journal: NATURE, **1994**, V371, N6496 (SEP 29), P419-423
ISSN: 0028-0836
Language: ENGLISH Document Type: ARTICLE
Abstract: CYCLIN-dependent kinase 5 (Cdk5) was originally isolated through its structural homology to human Cdc2(1), a key regulator of cell-cycle progression(2-6). In tissue samples from adult mice, Cdk5 protein is found at the highest level in brain, at an intermediate level in testis, and at low or undetectable levels in all other tissues, but brain is the only tissue that shows Cdk5 histone H1 kinase activity(7). No equivalent kinase activity has been found in tissue culture cell lines despite high levels of Cdk5 This raised the possibility that a Cdk5 regulatory subunit was responsible for the activation of Cdk5 in brain. Here we describe the cloning and characterization of a regulatory subunit for Cdk5 known as p35. p35 displays a neuronal cell-specific pattern of expression, it associates physically with Cdk5 in vivo and activates the Cdk5 kinase. p35 differs from the mammalian cyclins and thus represents a new type of **regulatory** subunit for **cyclin-dependent kinase** activity.

Title: P35 IS A NEURAL-SPECIFIC **REGULATORY** SUBUNIT OF **CYCLIN-DEPENDENT KINASE**-5
, **1994**

12930737 PMID: 8601310

Crystal structure and mutational analysis of the **human** CDK2 kinase complex with cell cycle-regulatory protein CksHs1.

Bourne Y; Watson M H; Hickey M J; Holmes W; Rocque W; Reed S I; Tainer J A

Department of Molecular Biology, Scripps Research Institute, La Jolla, California, 92037, USA.

Cell (UNITED STATES) Mar 22 **1996**, 84 (6) p863-74, ISSN 0092-8674 Journal Code: 0413066

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The 2.6 Angstrom crystal structure for **human cyclin-dependent kinase** 2(CDK2) in complex with CksHs1, a **human** homolog of essential **yeast** cell cycle-regulatory proteins suc1 and **Cks1** , reveals that CksHs1 binds via all four beta strands to the kinase C-terminal lobe. This interface is biologically critical, based upon mutational analysis, but far from the CDK2 N-terminal lobe, cyclin, and regulatory phosphorylation sites. CDK2 binds the Cks single domain conformation and interacts with conserved hydrophobic residues plus His-60 and Glu-63 in their closed beta-hinge motif conformation. The beta hinge opening to form the Cks beta-interchanged dimer sterically precludes CDK2 binding, providing a possible mechanism regulating CDK2-Cks interactions. One face of the complex exposes the sequence-conserved phosphate-binding region on Cks and the ATP-binding site on CDK2, suggesting that CKs may target CDK2 to other phosphoproteins during the cell cycle.

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Set     Items   Description
S1         85   SURVIVIN(5N)BIND?
S2         42   RD (unique items)
S3          9   S2 AND PY<=2001
S4      25728   CYCLIN(W)DEPENDENT(5N) KINASE
S5     105149   REGULAT?(5N)(DOMAIN?? OR REGION?? OR SUBUNIT?? OR AREA??)
S6        915   S4 AND S5
S7        208   CKS1 OR CKS2
S8        111   RD (unique items)
S9         45   S8 AND PY<2001
S10         4   S6 AND S7
S11         2   RD (unique items)
S12        45   S9 AND PY<=2001
S13        45   RD (unique items)
S14       586   S6 AND PY<2001
S15         0   CYCLIN(W)DEPEDENT(W)KINASE(2N)REGULAT?
S16      2013   CYCLIN(W)DEPENDENT(2N)KINASE(2N)REGULAT?
S17   3695017   DOMAIN?? OR REGION??
S18       370   S16 AND S17
S19       214   RD (unique items)
S20       155   S19 AND PY<=2001
S21       115   S20 AND PY<2000
S22    379168   CONJUGAT? OR CHIMER?
S23         3   S21 AND S22
? s regulat?(2n)(domain?? or region??)
         2231661  REGULAT?
          796123  DOMAIN??
         3095234  REGION??
     S24   46207  REGULAT?(2N)(DOMAIN?? OR REGION??)
? s s24 and s16
           46207  S24
            2013  S16
     S25      13  S24 AND S16
? rd
>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
     S26       9  RD (unique items)
? s s26 and py<=2001
Processing
               9  S26
        42056921  PY<=2001
     S27       9  S26 AND PY<=2001
? t s27/3,k,ab/1-9

 27/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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14013693   PMID: 9710585
  Deregulation of poly(A) polymerase interferes with cell growth.
  Zhao W; Manley J L
  Department of Biological Sciences, Columbia University, New York, New
York 10027, USA.
  Molecular and cellular biology (UNITED STATES)   Sep 1998,  18  (9)
  p5010-20,  ISSN 0270-7306   Journal Code: 8109087
  Contract/Grant No.: GM 28983; GM; NIGMS
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  Vertebrate poly(A) polymerase (PAP) conta
```